

***IN THE UNITED STATES PATENT AND TRADEMARK OFFICE***

Applicant: CEBON, et al.  
Title: In Vivo Efficacy of NY-ESO-1  
Plus Adjuvant  
Appl. No.: 10/573,753  
International  
Filing Date: 9/30/2004  
Examiner: Marianne Dibrino  
Art Unit: 1644  
Confirmation 3988  
Number:

**RESPONSE TO REQUEST FOR INFORMATION UNDER 37 CFR 1.105**

Mail Stop AF  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

This is a response to the Request for Information set forth in the Advisory Action mailed July 12, 2010, in the captioned patent application. As this is the first response to the Request for Information, the fee and certification requirements for the documents submitted herewith (listed on the accompanying Form PTO/SB/08) are waived, as stated at page 3 of the Advisory Action. Applicant respectfully requests that each listed document be considered by the Examiner and be made of record in the present application and that an initialed copy of Form PTO/SB/08 be returned in accordance with MPEP §609.

## RESPONSE TO REQUEST FOR INFORMATION

The Patent Office has made a Request for Information related to the Cebon abstract presented at the 2002 annual meeting of the American Society of Clinical Oncology (the "Cebon abstract"). In response, Applicant provides the following information, which is based on information received from Dr. Johnathan Cebon, an inventor of the application and coauthor of the abstract, slides and poster from the ASCO 2002 annual meeting.

The Cebon abstract was part of a poster displayed at the ASCO 2002 annual meeting, which was held in Orlando, Florida. A copy of the poster is submitted herewith as part of the response to the Request for Information. There was no oral presentation of the poster, abstract or underlying subject matter at the ASCO 2002 annual meeting. The slides were part of a "virtual meeting" accessible via the ASCO website to virtual meeting registrants.

Specific answers to the questions set forth in the Request for Information are provided below.

- **A statement describing the amount of ISCOM adjuvant in each of the different protein dosage administrations, i.e., for 10 ug, 30 ug and 100 ug of NY-ESO-1.**

The in vivo trial presented in the Cebon abstract and slides corresponds to that described in Example 1 of the application. Thus, the amount of protein and adjuvant used were:

Dose level A = 10  $\mu$ g NY-ESO-1 protein in 12  $\mu$ g ISCOM

Dose level B = 30  $\mu$ g of NY-ESO-1 protein in 36  $\mu$ g ISCOM

Dose level C = 100  $\mu$ g of NY-ESO-1 protein in 120  $\mu$ g ISCOM

Dose level D = 100  $\mu$ g NY-ESO-1 protein without ISCOM

The specification as filed includes a clerical error with regard to dose level B, in that paragraph [0023] states that 36  $\mu$ g protein was used, where it was 30  $\mu$ g protein. The correct protein dose is indicated in Figures 1 and 2. This error is being corrected in the response submitted herewith.

- **A statement describing if reducing the risk of relapse was presented/discussed during the slide presentation.**

As noted above, there was no oral presentation or discussion of the subject matter underlying the Cebon abstract and slides at the ASCO 2002 annual meeting. Moreover, relapse data were not available at the time. Thus, there was no presentation of relapse data at the ASCO 2002 annual meeting.

- **A statement describing all of the data that was presented and how that data is related to the data of the instant specification.**

All of the data presented at the ASCO 2002 annual meeting are set forth in the Cebon abstract and slides already of record, and the poster submitted herewith (which is cumulative of the abstract and slides). As noted above, the in vivo trial presented in the Cebon abstract corresponds to that described in Example 1 of the application. Other data presented correspond to Example 2, Example 3 (Figure 2 but not Figure 3) and Example 4 of the application. The results reported in Examples 5-17 of the specification were not presented at the ASCO 2002 annual meeting.

- **In response to this request, Applicant is also requested to furnish:**

**A statement describing additional presentations and/or abstracts presented by Applicant at scientific meetings wherein data pertinent to the subject matter was disclosed, and the contents of such disclosures, if such disclosures in fact occurred.**

There were two additional presentations of subject matter presented at the ASCO 2002 annual meeting and disclosed in the application, prior to the September 30, 2004 filing date of the PCT application. The first was a presentation at the December 2002 Australian Society of Immunology. A copy of the slides from that presentation are submitted herewith. The second was an invited seminar given at Auckland University in July 2003. A copy of the slides from that seminar are submitted herewith. These presentations include some additional data beyond that presented at the ASCO 2002 annual meeting, relating to CD4 and CD8 T

cell responses, the use of DCs to generate T cells, and immunohistochemistry experiments. However, again, no relapse data or patient survival data were presented.

As this response replies to each requirement for information giving the information required, it is believed to be a complete response to the Requirement for Information under 37 CFR 1.105.

Respectfully submitted,

Date August 2, 2010

FOLEY & LARDNER LLP  
Customer Number: 22428  
Telephone: (202) 295-4094  
Facsimile: (202) 672-5399

By Courtenay C. Brinckerhoff

Courtenay C. Brinckerhoff  
Attorney for Applicant  
Registration No. 37,288



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Substitute for form 1449/PTO				<b>Complete if Known</b>	
<b>INFORMATION DISCLOSURE STATEMENT BY APPLICANT</b>  Date Submitted: August 2, 2010 <i>(use as many sheets as necessary)</i>				Application Number	10/573,753
				Filing Date	9/30/2004
				First Named Inventor	Jonathan CEBON
				Art Unit	1644
				Examiner Name	Marianne Dibrino
Sheet	1	of	1	Attorney Docket Number	029860-0145

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. <sup>1</sup>	Document Number Number-Kind Code <sup>2</sup> (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

UNPUBLISHED U.S. PATENT APPLICATION DOCUMENTS					
Examiner Initials*	Cite No. <sup>1</sup>	U.S. Patent Application Document Serial Number-Kind Code <sup>2</sup> (if known)	Filing Date of Cited Document MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. <sup>1</sup>	Foreign Patent Document Country Code <sup>3</sup> -Number <sup>4</sup> -Kind Code <sup>5</sup> (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Documents	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T <sup>6</sup>

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. <sup>1</sup>	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.) date, page(s), volume-issue number(s), publisher, city and/or country where published.	T <sup>6</sup>
	A1	CEBON ET AL., "A phase I study of NY-ESO-1 ISCOM® in patients with NY-ESO-1 positive cancers and minimal residual disease," from ASCO Annual Meeting, 2002, Orlando, Florida (Poster).	
	A2	CEBON ET AL., "A phase I study of NY-ESO-1 ISCOM® in patients with NY-ESO-1 positive cancers and minimal residual disease," slides presented at Australian Society of Immunology, December 2002.	
	A3	CEBON ET AL., "Cancer Vaccination," slides presented at seminar given at Auckland University, July 2003.	

Examiner Signature	Date Considered
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\*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 See Kinds Codes of USPTO Patent Documents at [www.uspto.gov](http://www.uspto.gov) or MPEP 901.04. 3 Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). 4 For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 5 Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. 6 Applicant is to place a check mark here if English language Translation is attached.

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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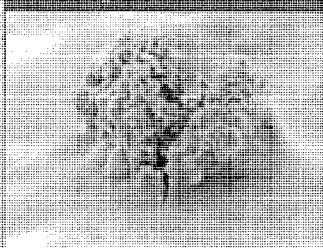
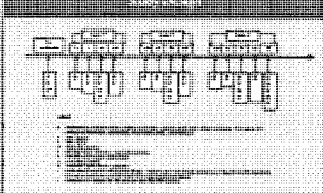
## A phase I study of NY-ESO-1 ISCOM® in patients with NY-ESO-1 positive cancers and minimal residual disease

[illegible][illegible]

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Treatment Type	%	n
Malignant	47	154
Metastomized areas	23	30
Carcinoma	41	25
Metastatic	20	10
TCC	25	4
Carcinoma	50	0
Metastatic	47	11

1. The first step in the process is to identify the problem or issue that needs to be addressed. This involves gathering information and understanding the context of the problem.

[illegible][illegible]

- 1. Tumor cell
  - 2. Tumor
  - 3. MYELOID LEUKEMIA
    - 4. paraneoplastic
      - 5. Dementia, Atrial Fibrillation
      - 6. Myeloperoxidase (MPO) antibodies for paraneoplastic immunological assays
    - 7. Anti-MPO, ANCA
  - 8. Anti-MPO associated to 28 patients
    - 9. 12 MPO-ANCA (32 patients), 16 MPO-ANCA (16 patients)
- 2. Protein alone
  - 3. MPO associated to 50 patients
  - 4. 14 MPO-ANCA (32 patients), 16 MPO-ANCA (16 patients)

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- EffT cells secrete IFN- $\gamma$  and IL-2, promote infest
- Antitumor (cytotoxic) CTLs
- CD8+ T cells
  - Tumor-infiltrating lymphocytes
  - Cytotoxic yFas producing CD8+ T cells
- Effort - inhibits proliferation of tumor cells
- Always under development
- CD8+ T cells proliferation, apoptosis, senescence
- Clinical outcome - not all

1997

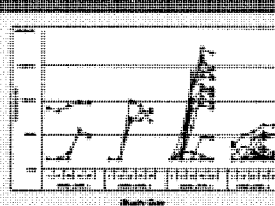
- Kin-280 + 100000 units used liberally
- Most cultures require more (grade 1 on 2)
- Grade 3 requires 1:1000000 and grade 4 1:100000
- Different grade 2 infections (2 or more positive) require 1:100000
- Fever
- Myalgia
- Headache
- Fl-like syndrome

## REFERENCES

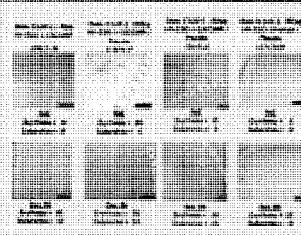
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Journal of Management Inquiry 20(4) 409-424

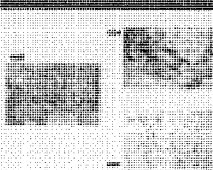
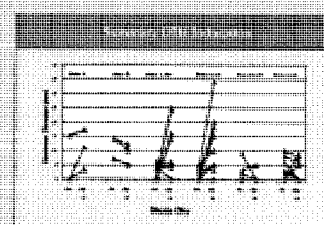
1. The first step in the process is to identify the problem or issue that needs to be addressed. This involves gathering information and understanding the context of the problem.



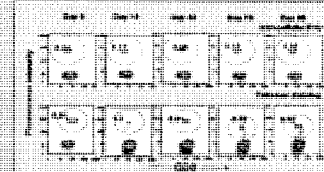
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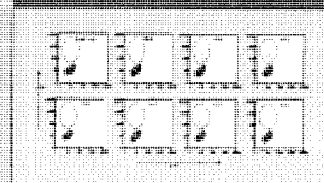
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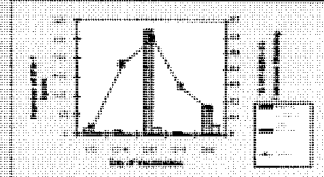
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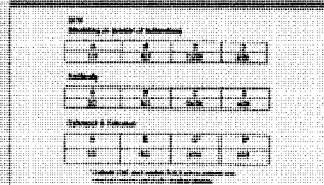
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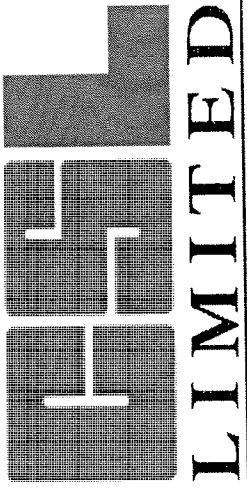
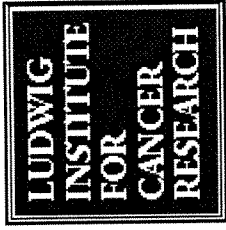


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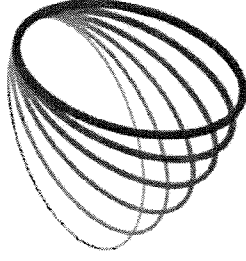
[illegible]

- [illegible]





**A phase I study of NY-ESO-1 ISCOM<sup>®</sup> in  
patients with NY-ESO-1 positive cancers and  
minimal residual disease**

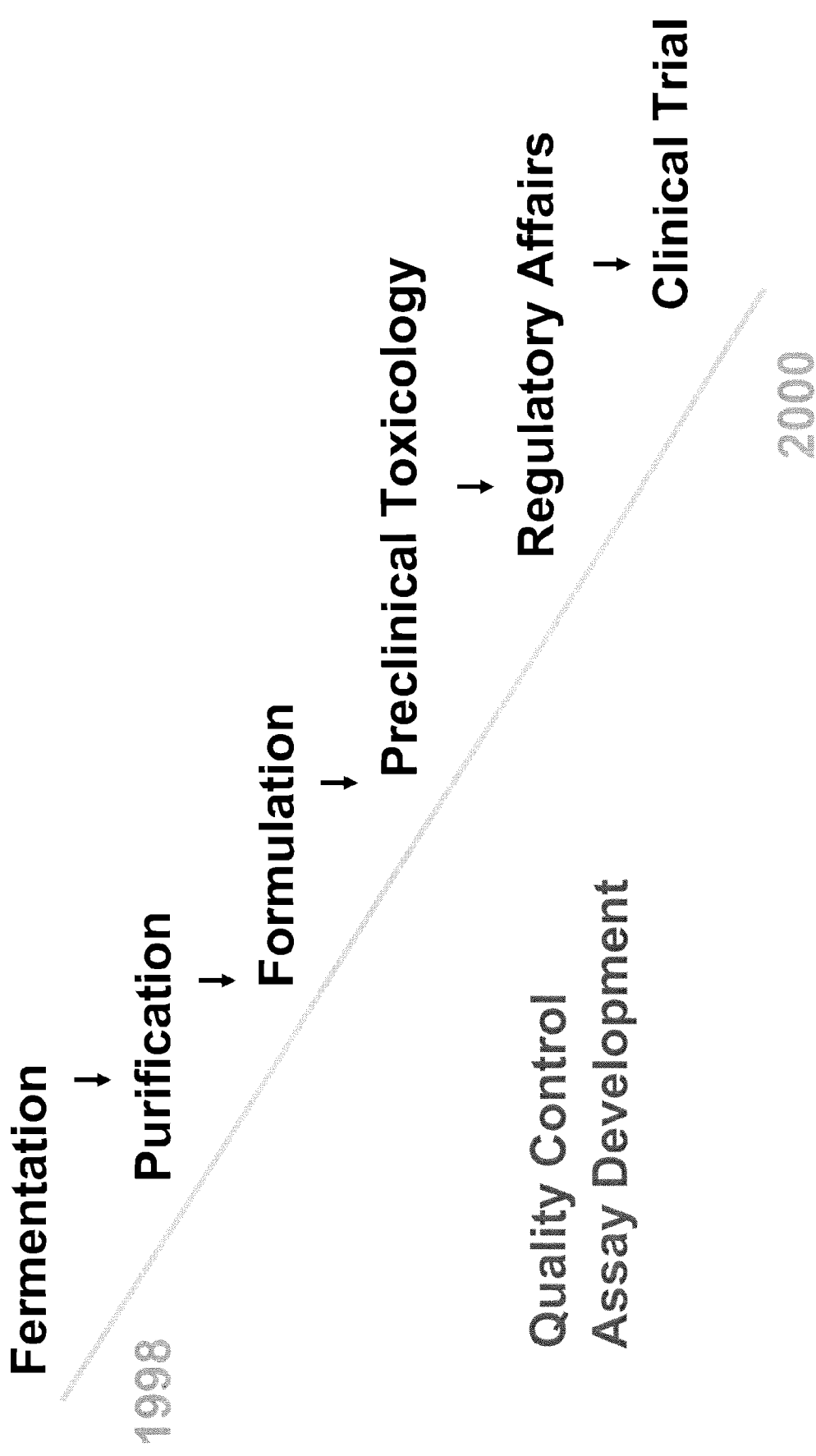


**CDCT**

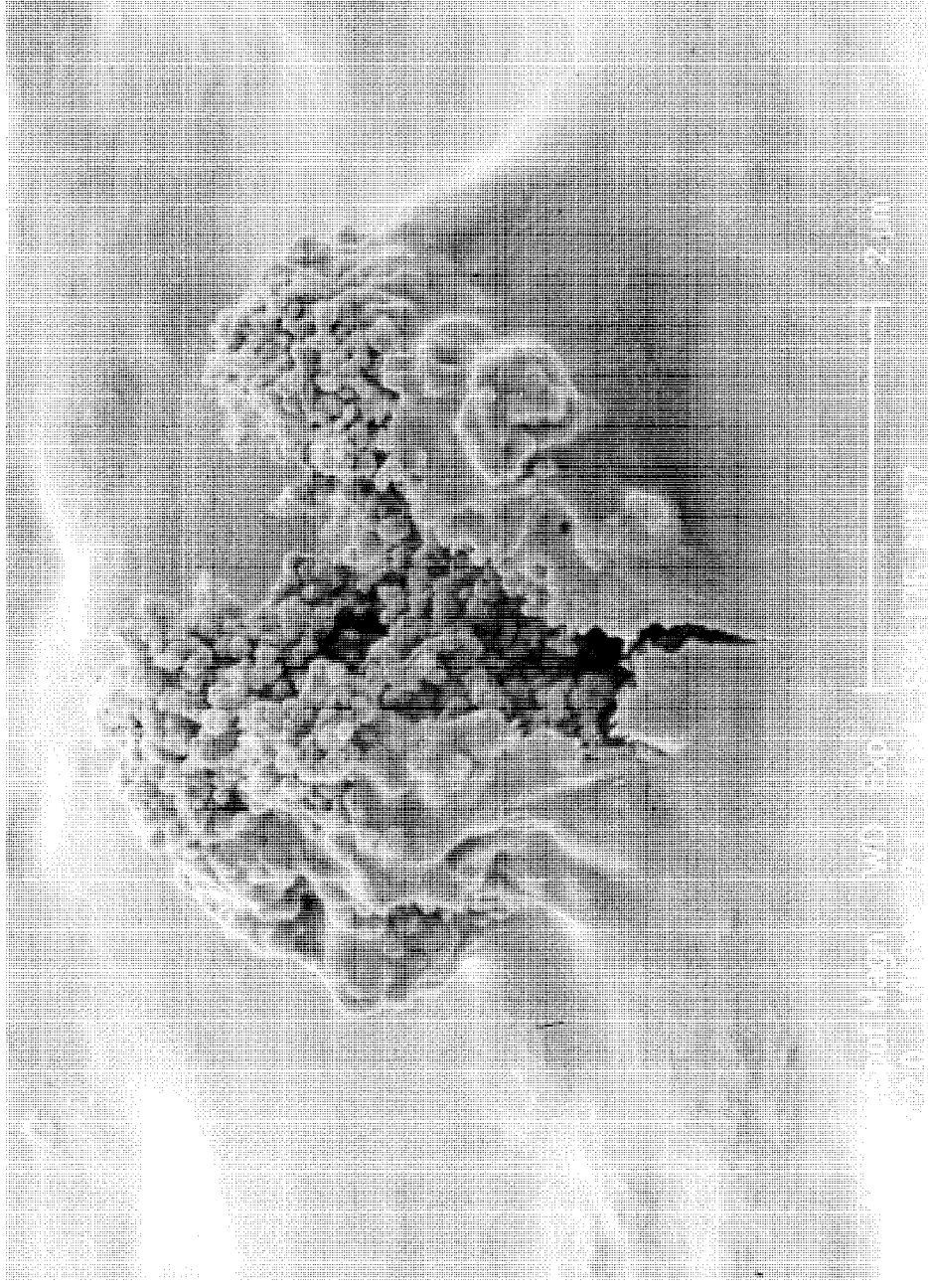
# mRNA expression NY-ESO-1

<u>Tumour Type</u>	<u>%</u>	<u>n</u>
Melanoma	41	154
Melanoma cell lines	33	30
Ca breast	40	25
HNSCC	20	10
TCC	25	4
Ca prostate	13	8
Hepatoma	47	31

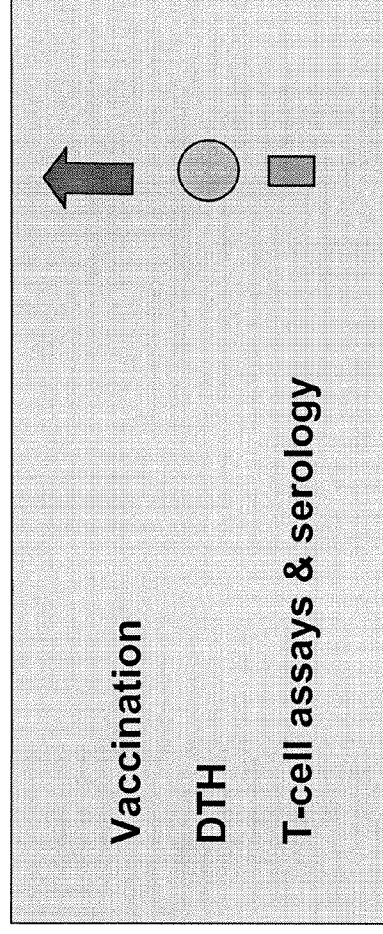
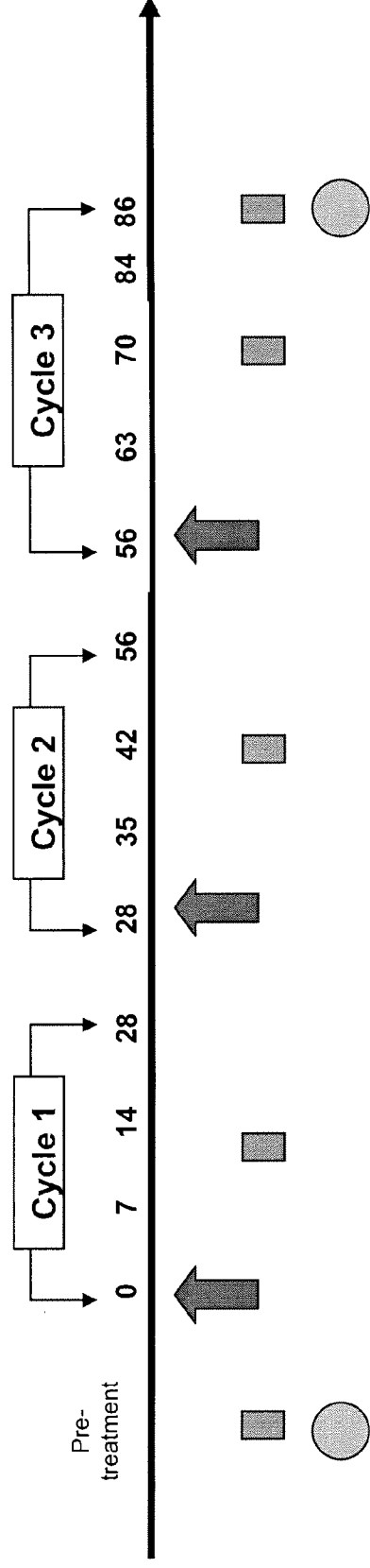
# Vaccine Production Timeline



# Scanning EM of NY-ESO-1 ISCOM®



# Study Design



# Patients

- Total 46
- 3 parts
  - 1 NY-ESO-1/ISCOM<sup>®</sup>
    - 3 pts/cohort
    - Dose levels A 10ug & B 30ug
    - Only HLA A2+ patients for purposes of immunological assays
  - 2 NY-ESO-1/ISCOM<sup>®</sup> - dose level C
    - Dose 100ug expanded to 20 patients
    - 10 HLA A2+ve (2 placebo), 10 HLA A2-ve (2 placebo)
  - 3 Protein alone - dose level D
    - 100ug expanded to 20 patients
    - 10 HLA A2+ve (2 placebo), 10 HLA A2-ve (2 placebo)



## Cancer Types

<b>On Study</b>	<b>51</b>
<b>Melanoma*</b>	<b>46</b>
<b>Ca Breast</b>	<b>3</b>
<b>TCC Bladder</b>	<b>1</b>
<b>Adenoid cystic carcinoma</b>	<b>1</b>

\*Stage II, III and IV resected

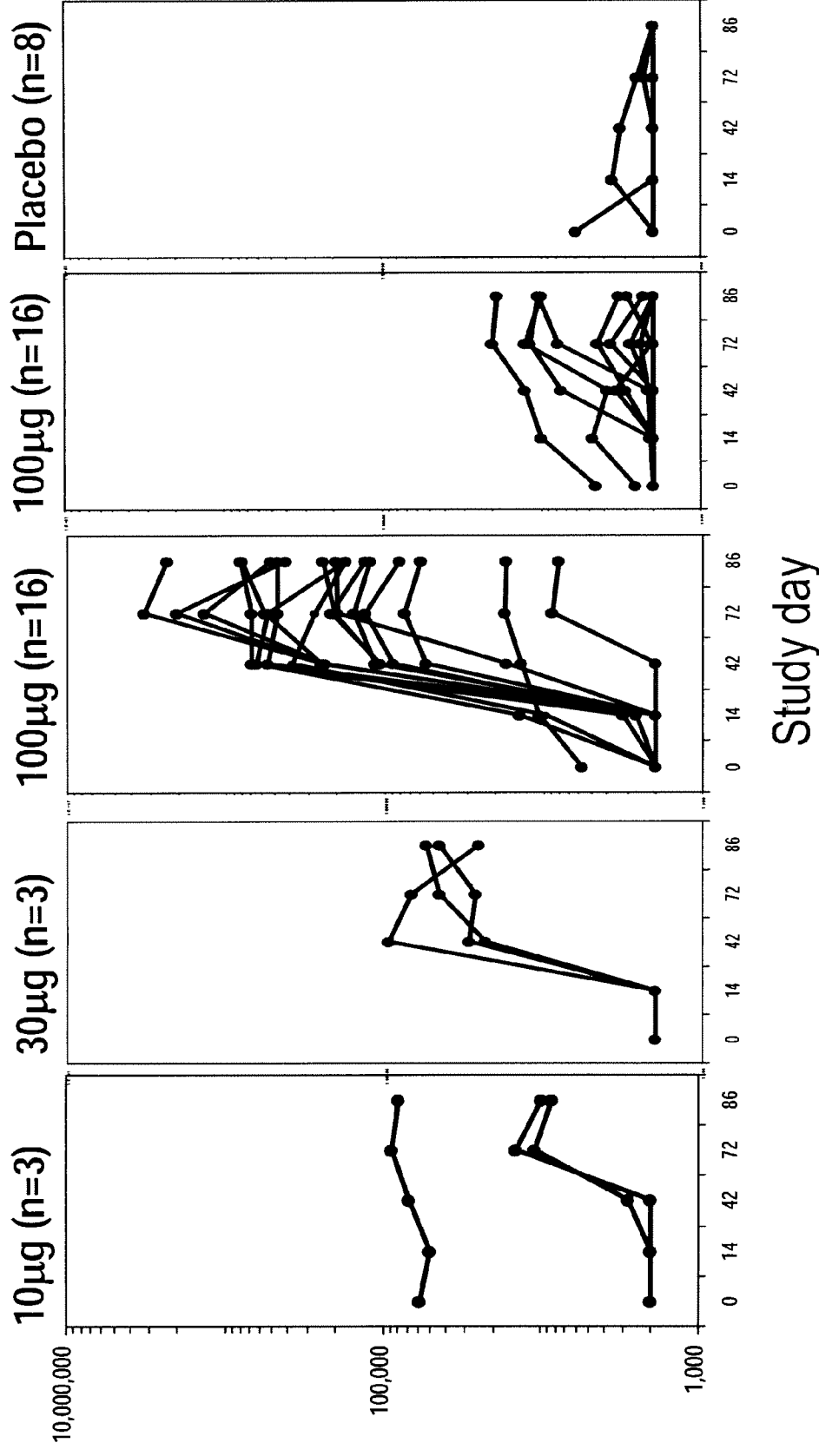
# Toxicity

- NY-ESO-1 ISCOM<sup>®</sup> was well tolerated
- Most adverse events were grade 1 or 2
- Grade 3 toxicities: injection site pain in 3/46
- Common grade 2 toxicities (2 or more patients)
  - Injection site pain
  - Fever
  - Myalgia
  - Headache
  - Flu-like symptoms

# Assays

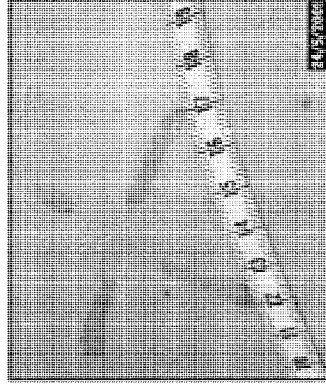
- DTH using NY-ESO-1 protein alone
- Antibody (capture ELISA)
- CD8+ T cells
  - Tetramer: SLLMWITQC
  - Cytospot: gIFN producing CD8+T Cells)
- Assays under development
  - *CD4+ T cells (DC & protein: cytokine secretion)*
  - *Class I epitopes - non HLA-A2*

# Antibody titre by cohort



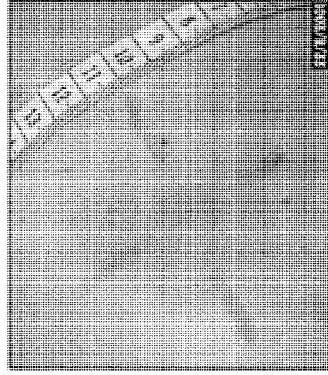
# Delayed-type Hypersensitivity: 1 $\mu$ g protein

Dose C (A2+) : 100 $\mu$ g  
NY-ESO-1-ISCIM® /  
Placebo  
126/KLE



PRE

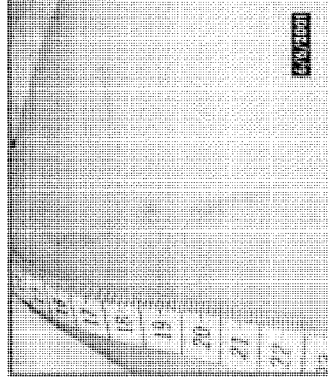
Erythema = 15  
Induration = 3



Day 86

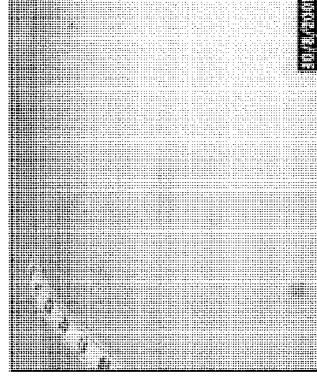
Erythema = 60  
Induration = 25

Dose D (A2-) : 100 $\mu$ g  
NY-ESO-1 Protein /  
Placebo  
127/JSM



PRE

Erythema = 2  
Induration = 0

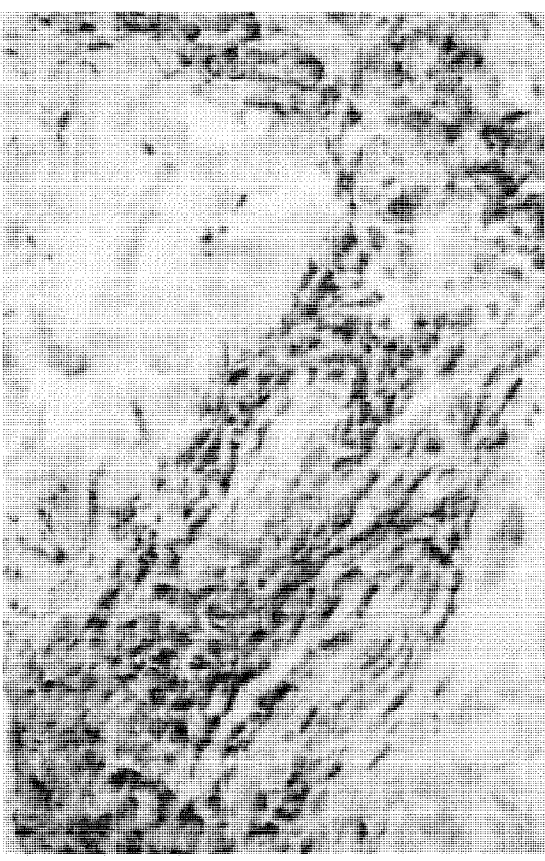


Day 86

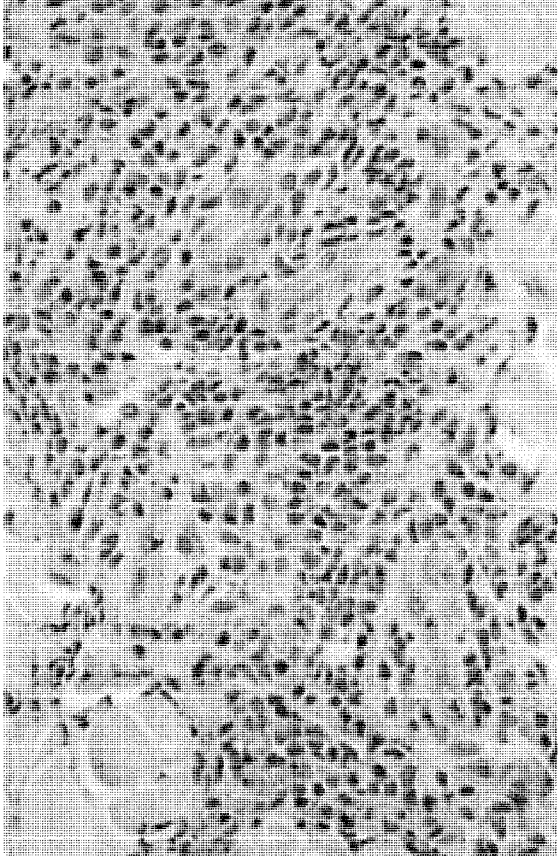
Erythema = 12  
Induration = 0

# DTH response to 1mg NY-ESO-1 protein

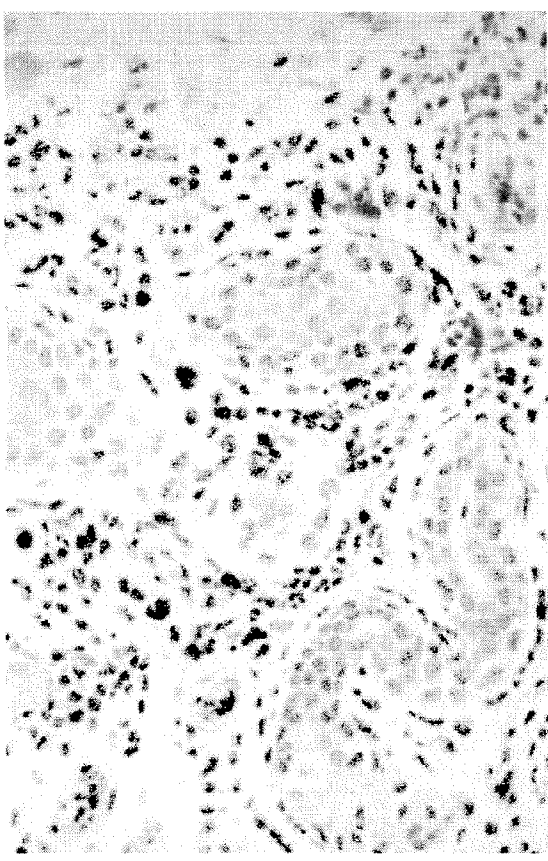
**CD4**



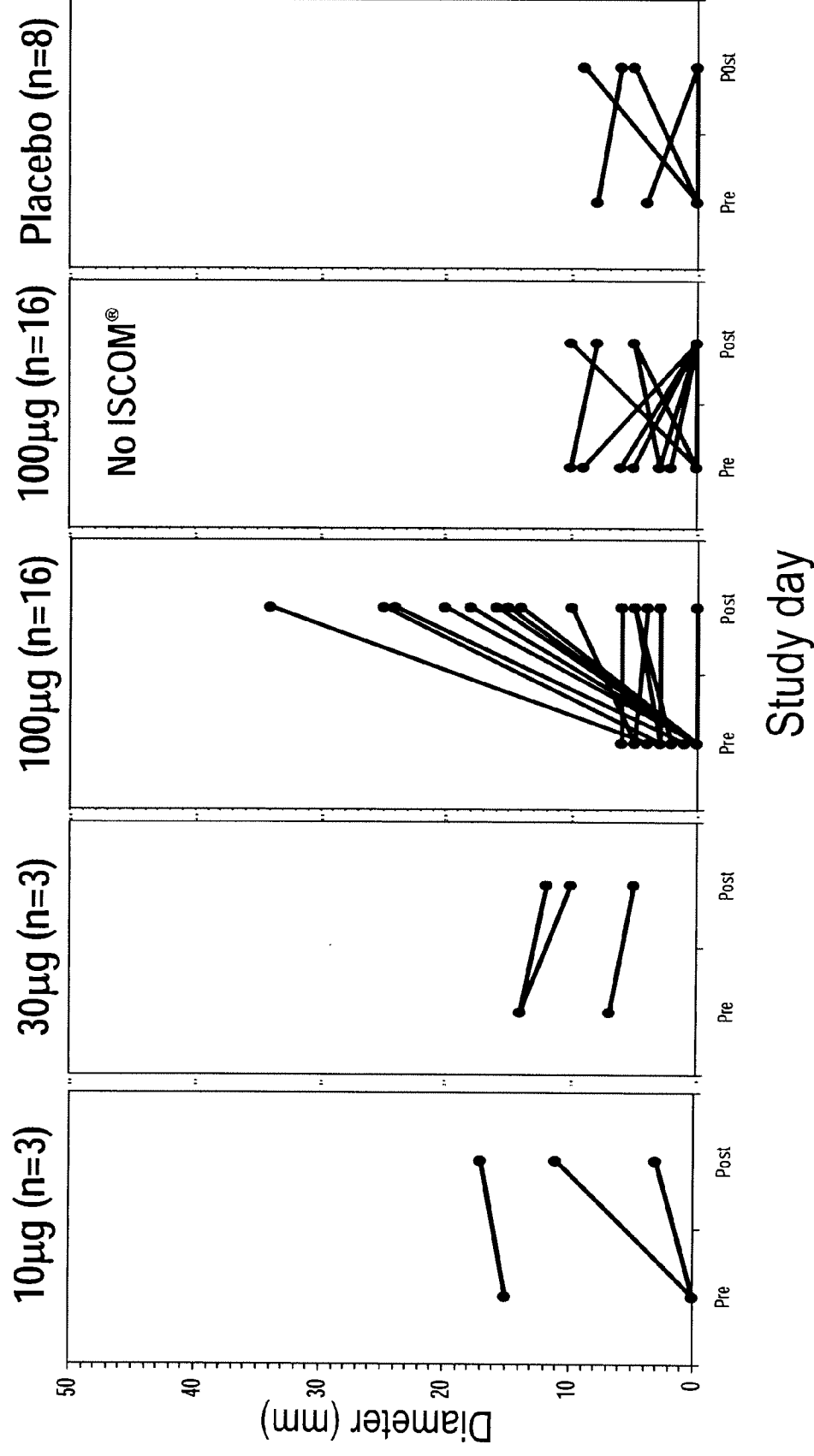
**H&E**



**CD8**

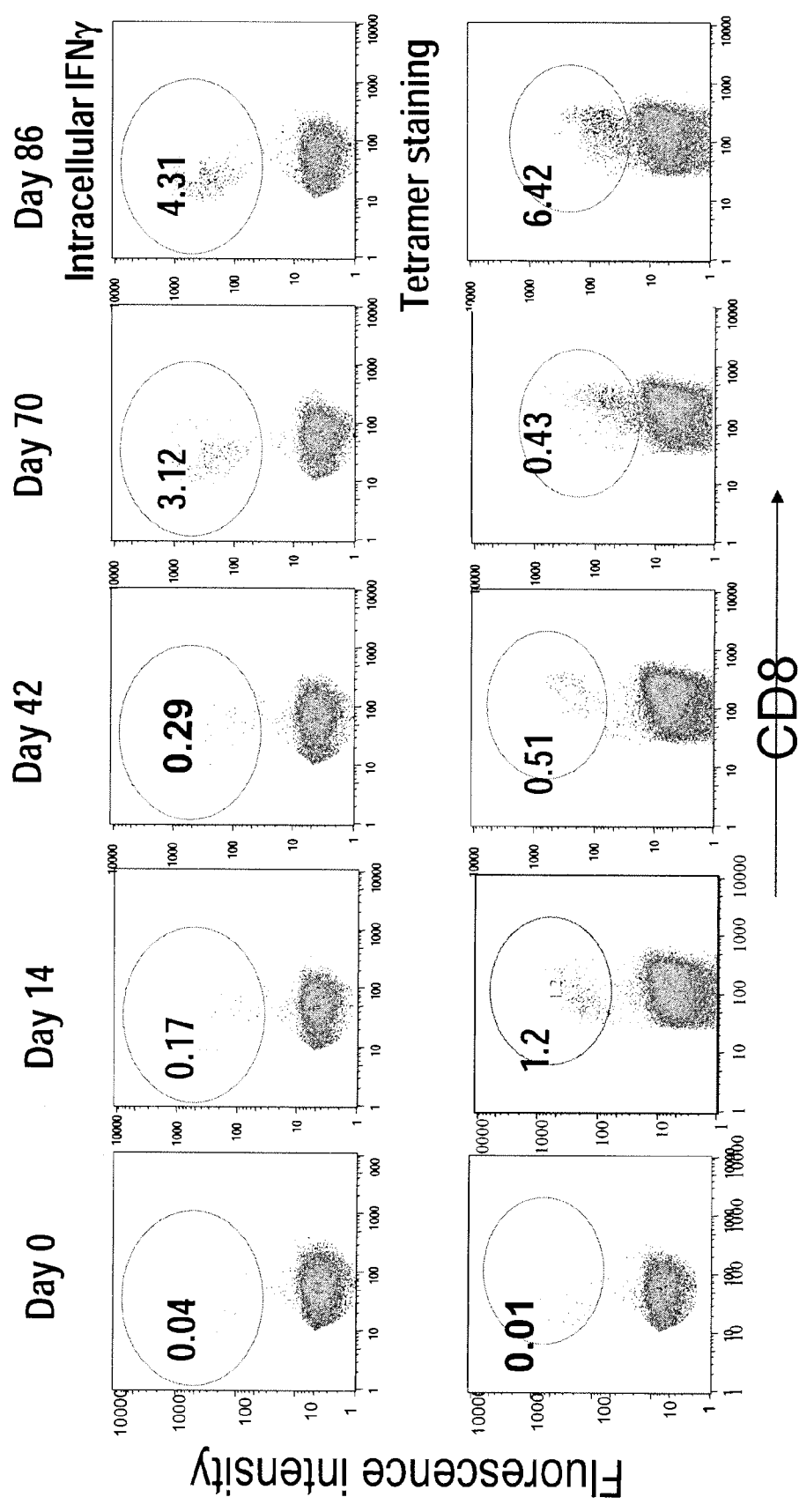


# DTH Induration by cohort



# T- cell response: $\gamma$ IFN production

## HLA A2+ pt (peptide SLLMWITQC)





# Summary Immunological Data

DTH (doubling or greater of induration)

A	B	C	D	Placebo
1/3	0/3	11/16	2/16	2/8

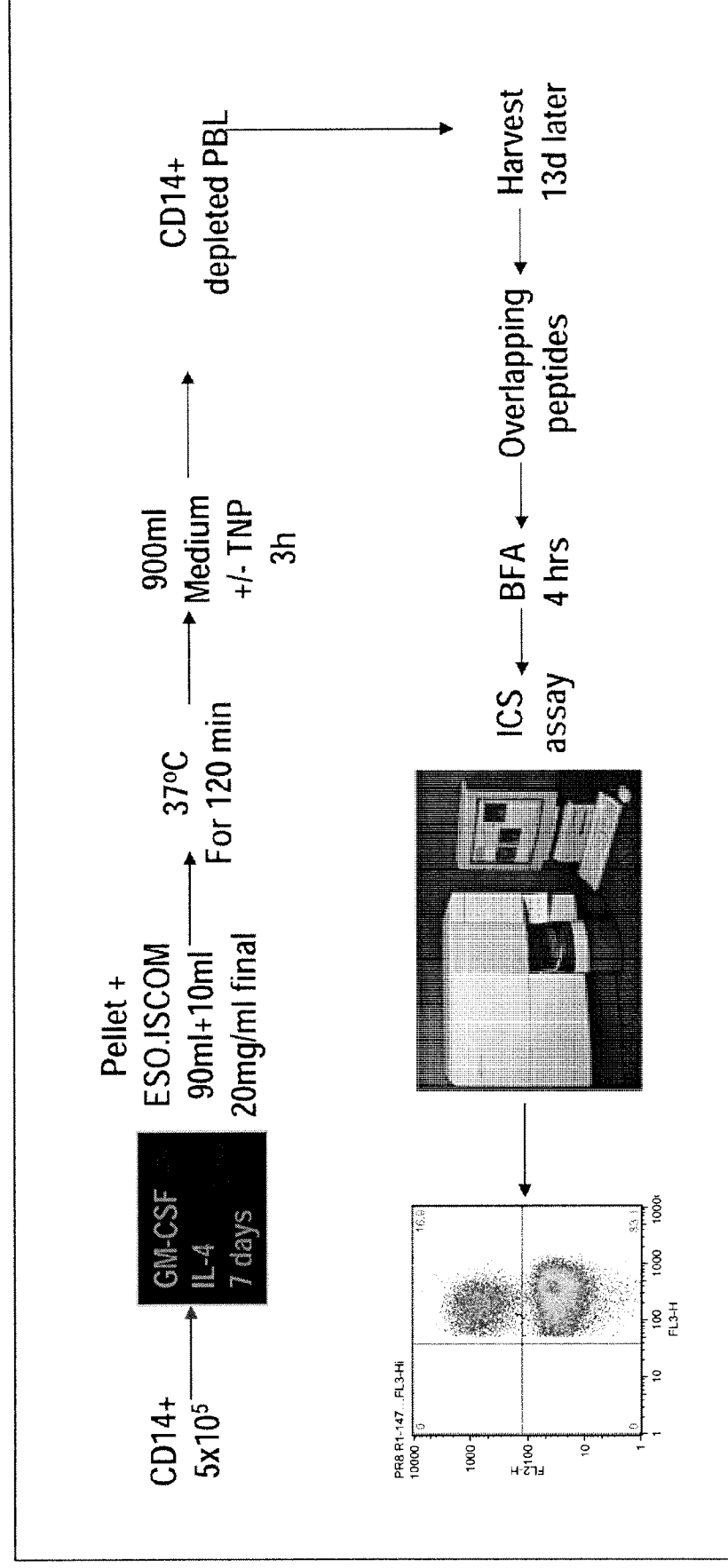
Antibody

A	B	C	D	Placebo
3/3	3/3	16/16	4/16	0/8

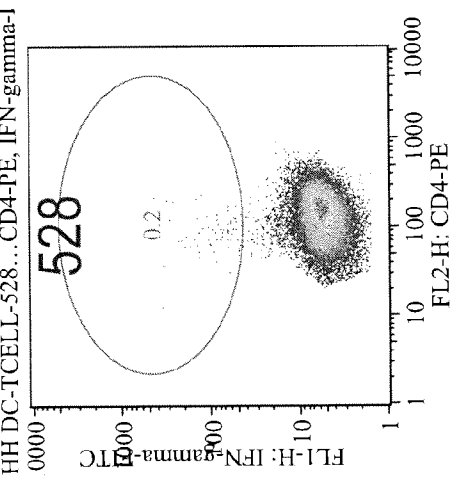
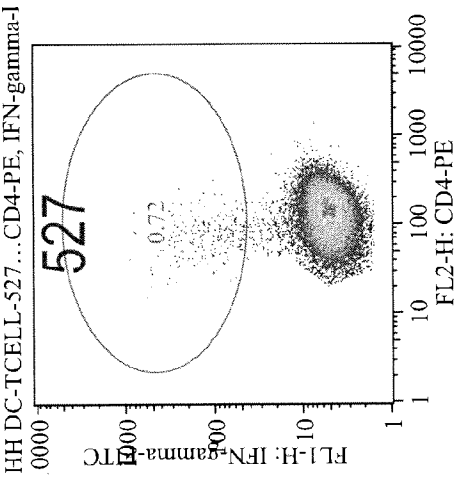
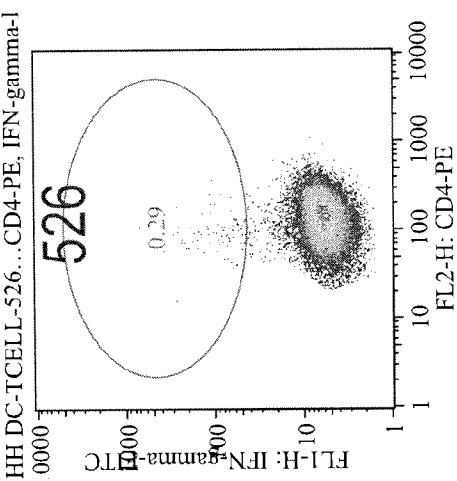
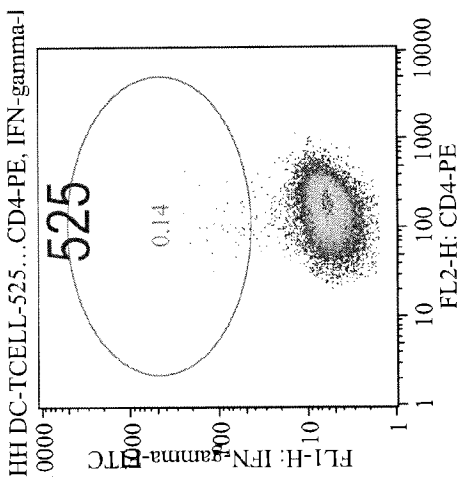
Cytospot & Tetramer

A	B	C	D	Placebo
1/3	0/3	3/8	1/8	0/4

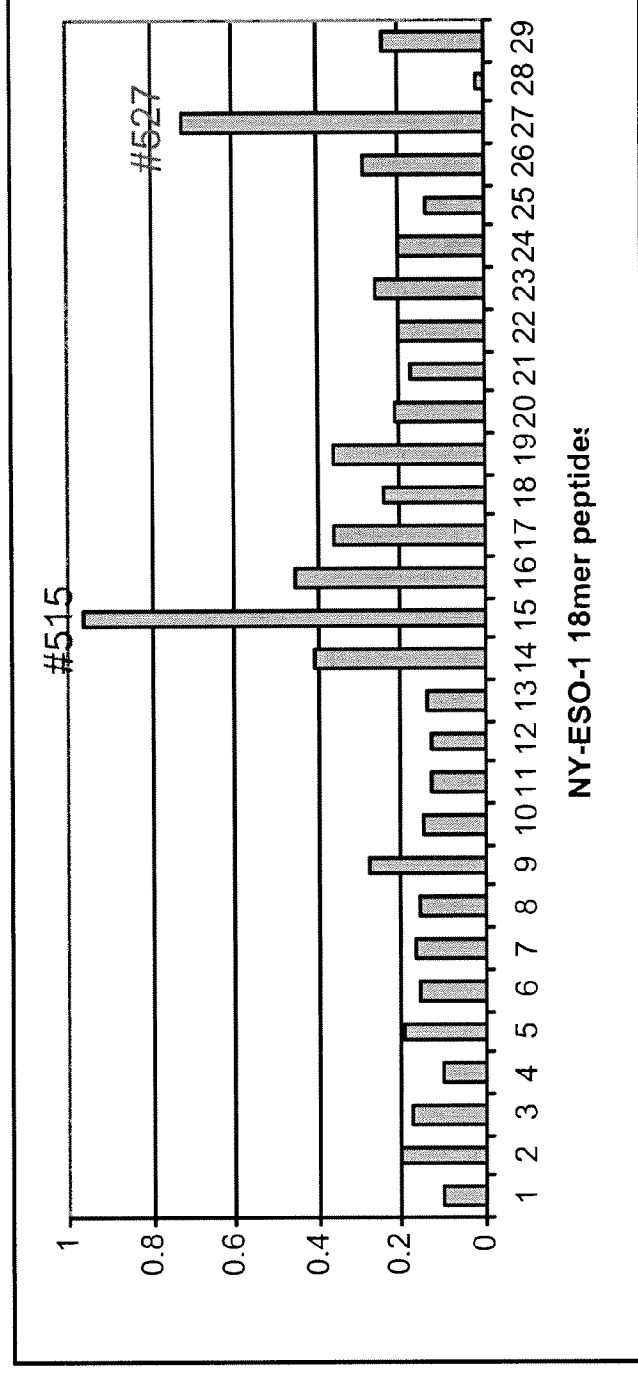
# Generation of NY-ESO-1 Specific T cells Using Tumor-Ag-loaded Autologous-DC



HH T cells generated with DC+ISCOM/NY-ESO-1 and screened with NY-ESO-1 18mer on day 13 after culture



T cells generated with DC+ISCOM/NY-ESO-1 and screened with 18mer peptides at day 13 after culture

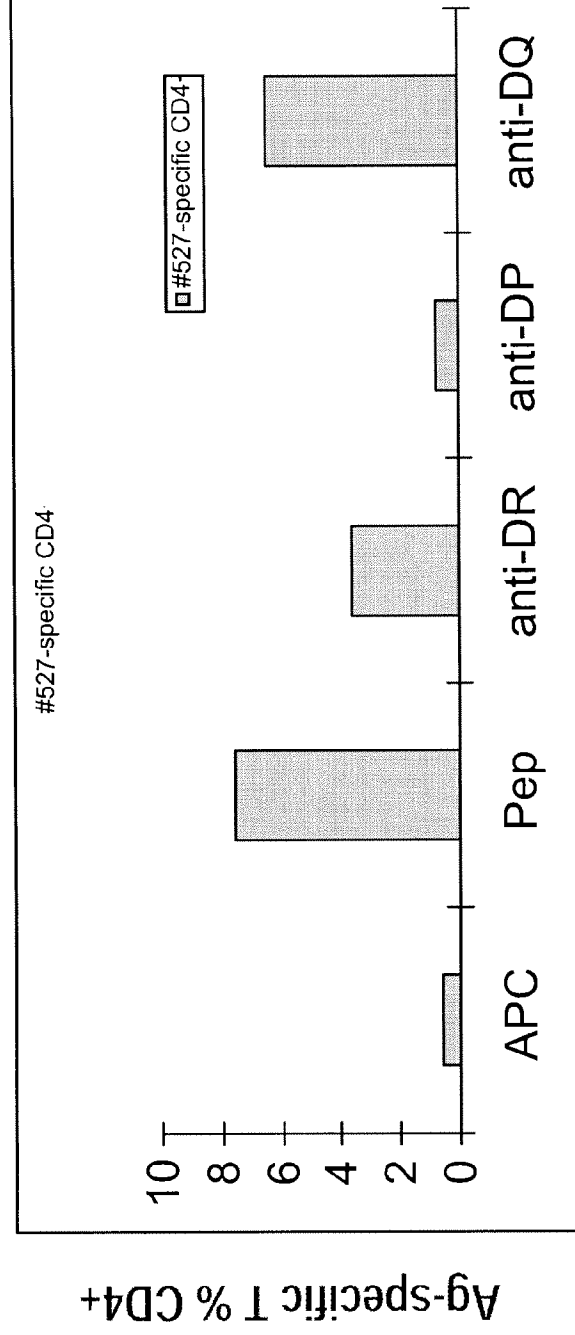


# Further characterisation of DC generated CD4 T cells

- Lines & clones established
- Antibodies
  - Anti DR, DP, DQ
- LCL lines
  - LCL auto: DR1, DR2, DP4
  - LCL 9080: DR1, ---, ---
  - LCL 9014: ---, DR2, ---
  - LCL T291: ---, DR2, DP4
  - LCL T282: ---, ---, DP4
- Tumor lines
  - NW38: DR1, ---, ---, NY-ESO-1(+)
  - LAR1a: ---, DR2, ---, NY-ESO-1(+)
  - SK-Mel 37: ---, ---, ---, NY-ESO-1(+)

# #527-specific CD4+ T cells are DP restricted

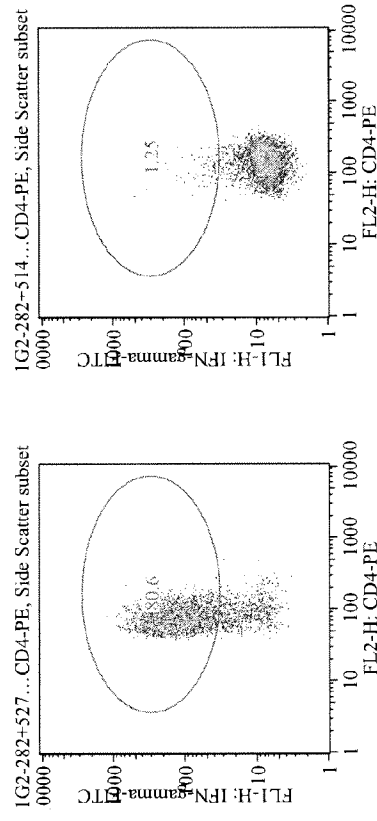
(DC stimulated then #527-pulsed BCL stimulated 2x)



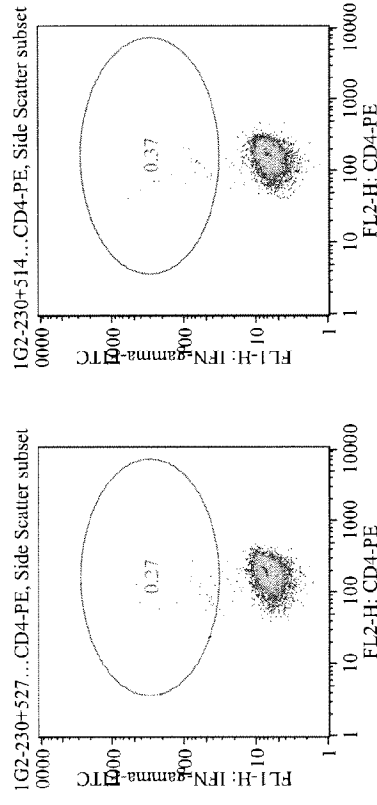
Treatments

# #527-specific T cells are DP4 restricted

DP4+  
LCL  
(282)

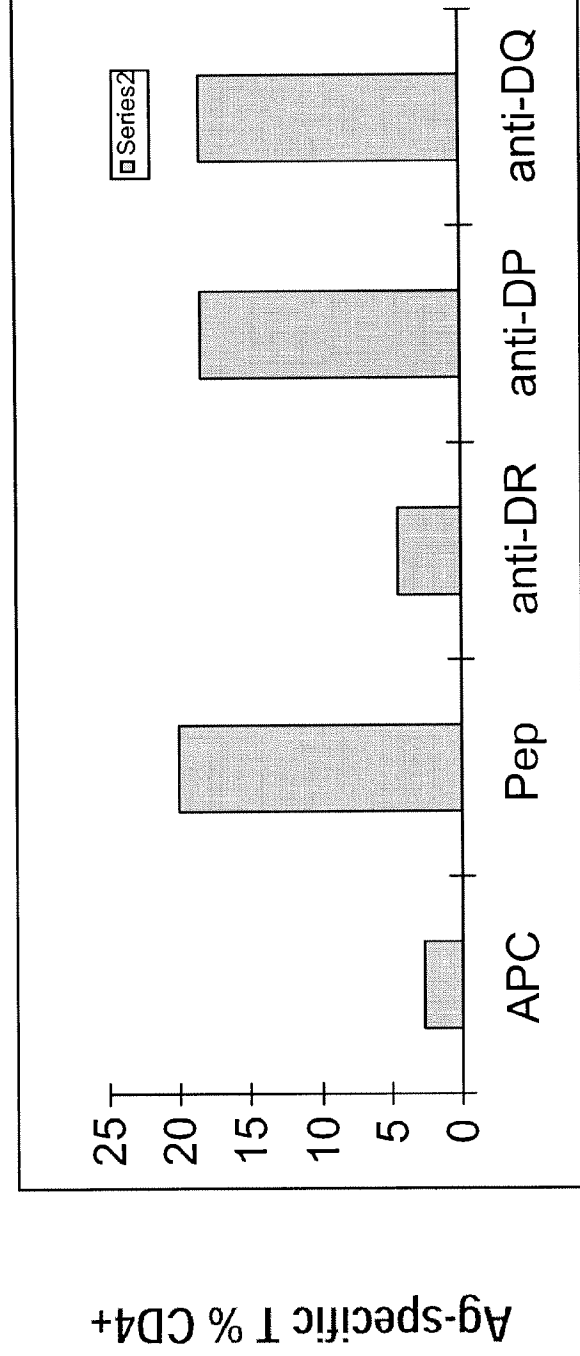


DP4-  
LCL  
(230)



# #515-specific CD4+ T cells are DR restricted

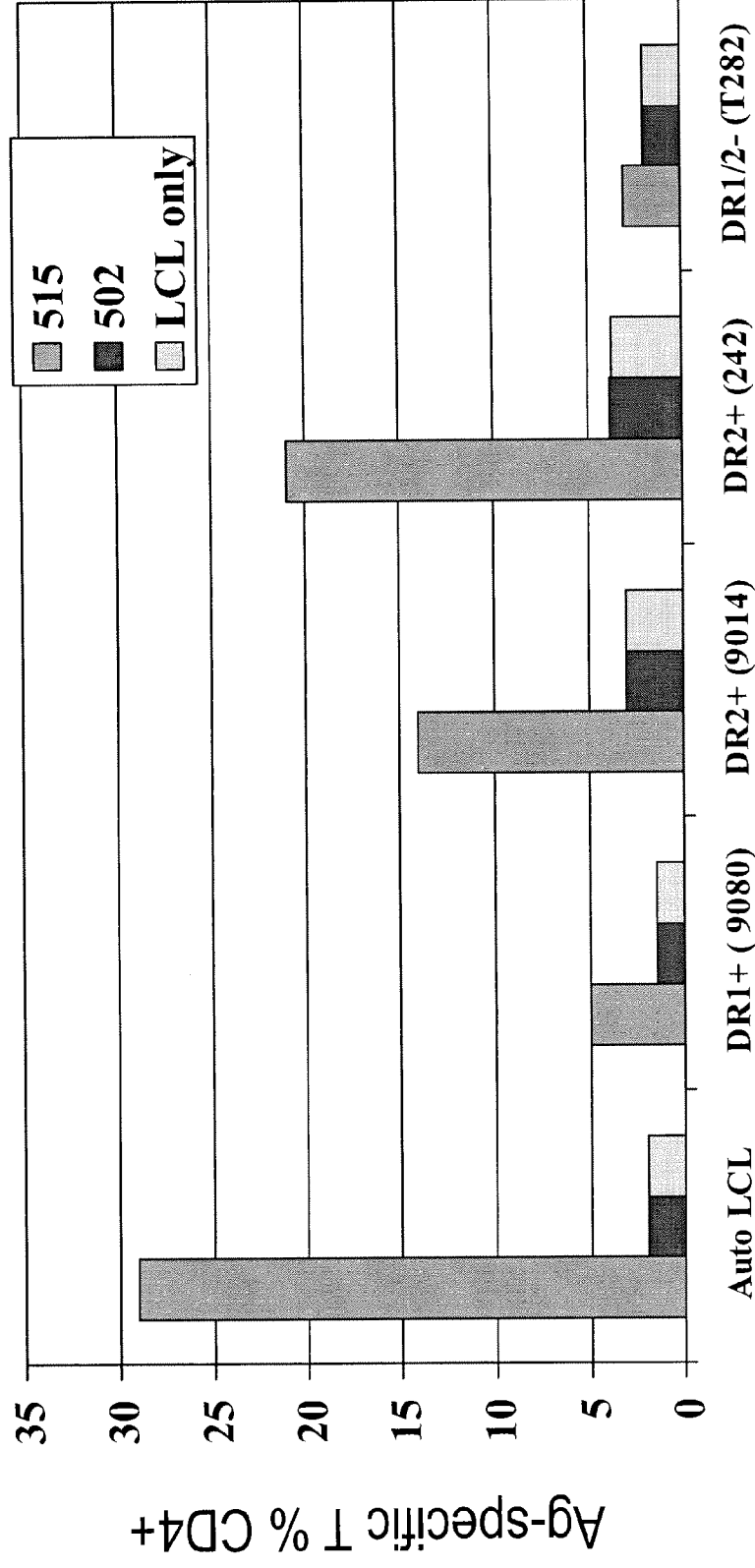
(DC stimulated then #515-pulsed BCL stimulated 2x)



Treatments

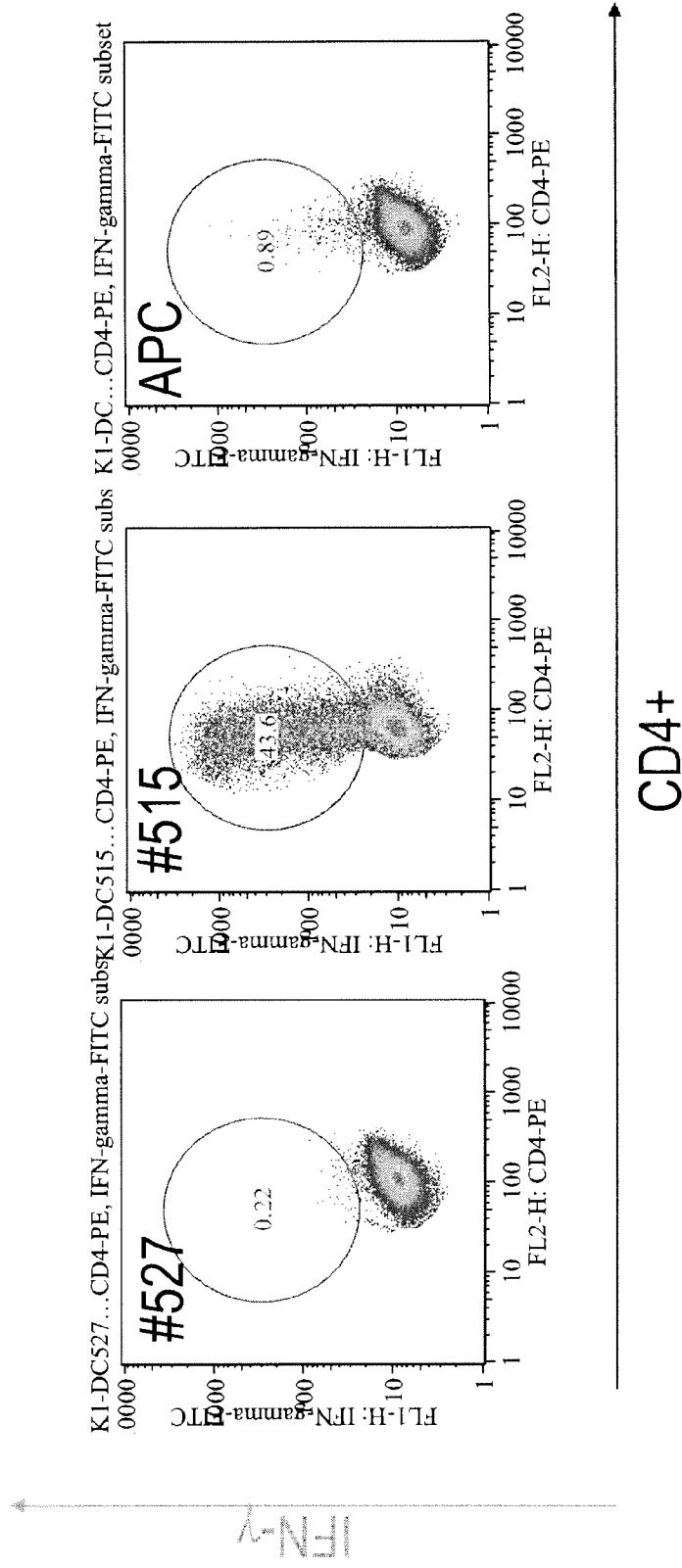


# DR restriction of #515-specific T cells ( #515 2xstimulations )

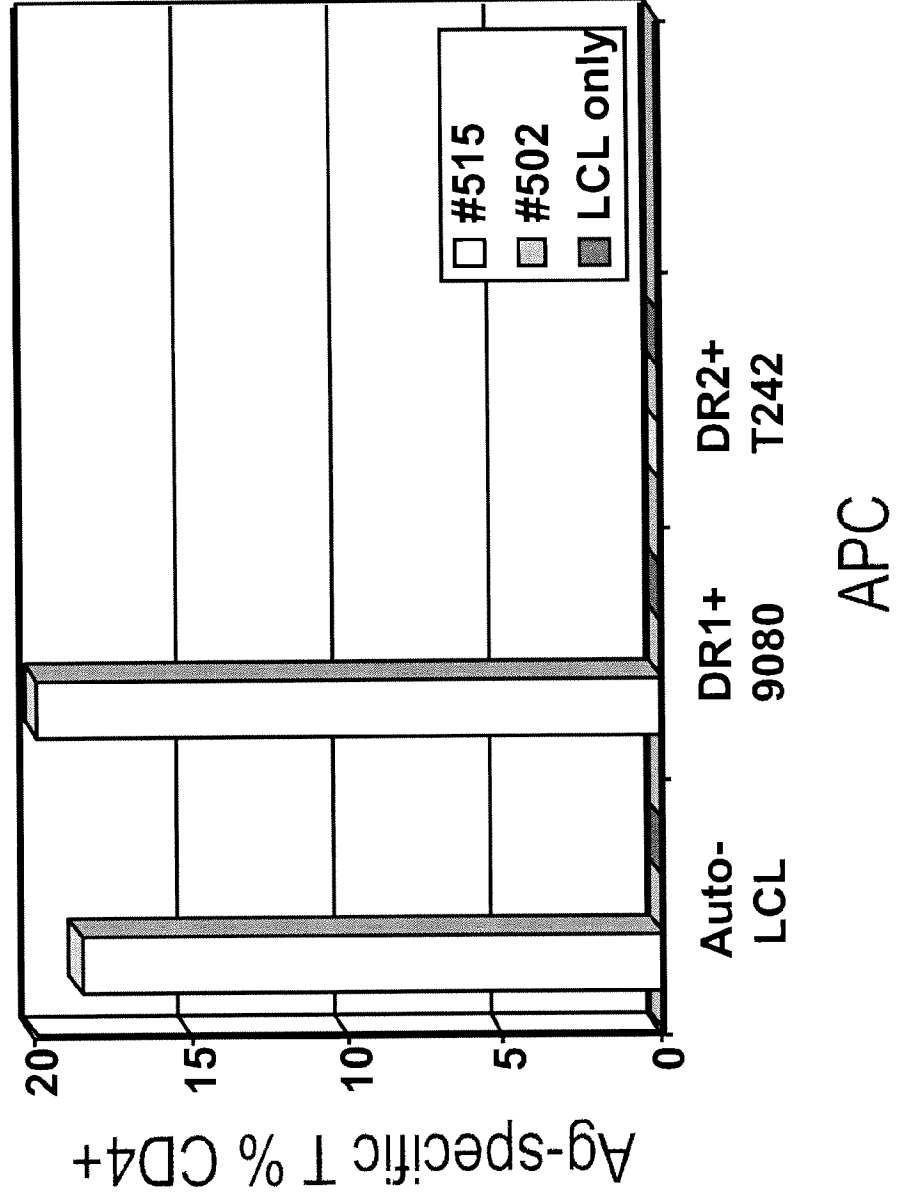


# #515-specific T clone K1

## generated with #515-pulsed DC



T clone K1 is DR1-restricted



# Conclusions

- NY-ESO-1 ISCOMs vaccinations were safely tolerated
- NY-ESO-1 ISCOMs generated both humoral & cellular responses
- ISCOM adjuvant generated superior DTH and antibody responses
- Cytospot assay in HLA A2+ve patients: positive in 1 level A pt (with prior Ab response), 3/8 level C patients and 1/8 level D patients.
- These responses were seen in patients with and without pre-existing antibody titres
- There was a good correlation between tetramer & cytospot data
- There is evidence of CD4 responses to 2 novel epitopes in first level C patient tested - this analysis is ongoing

# Acknowledgements

## ARMC - Oncology

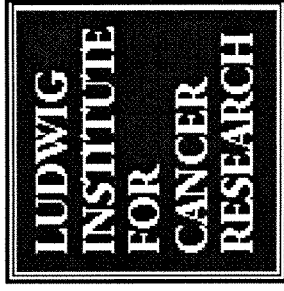
Ian Davis  
Mark Shackleton  
Phil Parente  
  
T-cell Laboratory  
Weisan Chen  
Qiyuan Chen  
Heather Jackson  
Eugene Maraskovsky  
Mark Rizkalla  
Tsin Tai  
Kelly-Anne Masterman  
  
CDCT  
Grant MacArthur  
Michael Green  
Richard Fox

## ARMC-BPF

Andrew Scott  
Roger Murphy  
Mike Rubira  
Glen Cartwright  
Jeff Rood  
  
CSL  
Simon Green  
Lena Miloradovic  
Andrew Cuthbertson  
Darryl Maher  
David Ryan  
Michael McNamara  
Debbie Drane

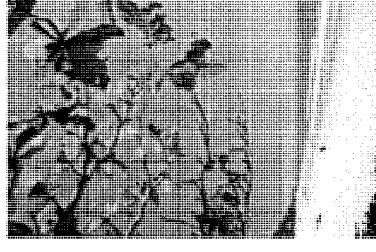
## Trials Centre

Wendy Hopkins  
Heather Goldie  
Sharen Gibbs  
Julie Newton  
  
New York  
Lloyd Old  
Eric Hoffman  
Gerd Ritter  
Sacha Gnjatic  
Yao Chen  
Lisa Stockert  
Lisa Pugliese  
  
Victorian Tissue Typing &  
Immunogenetics Service  
Brian Tait



# Cancer Vaccination

NY-ESO-1 as a model antigen



- Human cancers & immunity
- Melanoma
- NY-ESO-1 the antigen
- Vaccination with NY-ESO-1
- Clinical directions

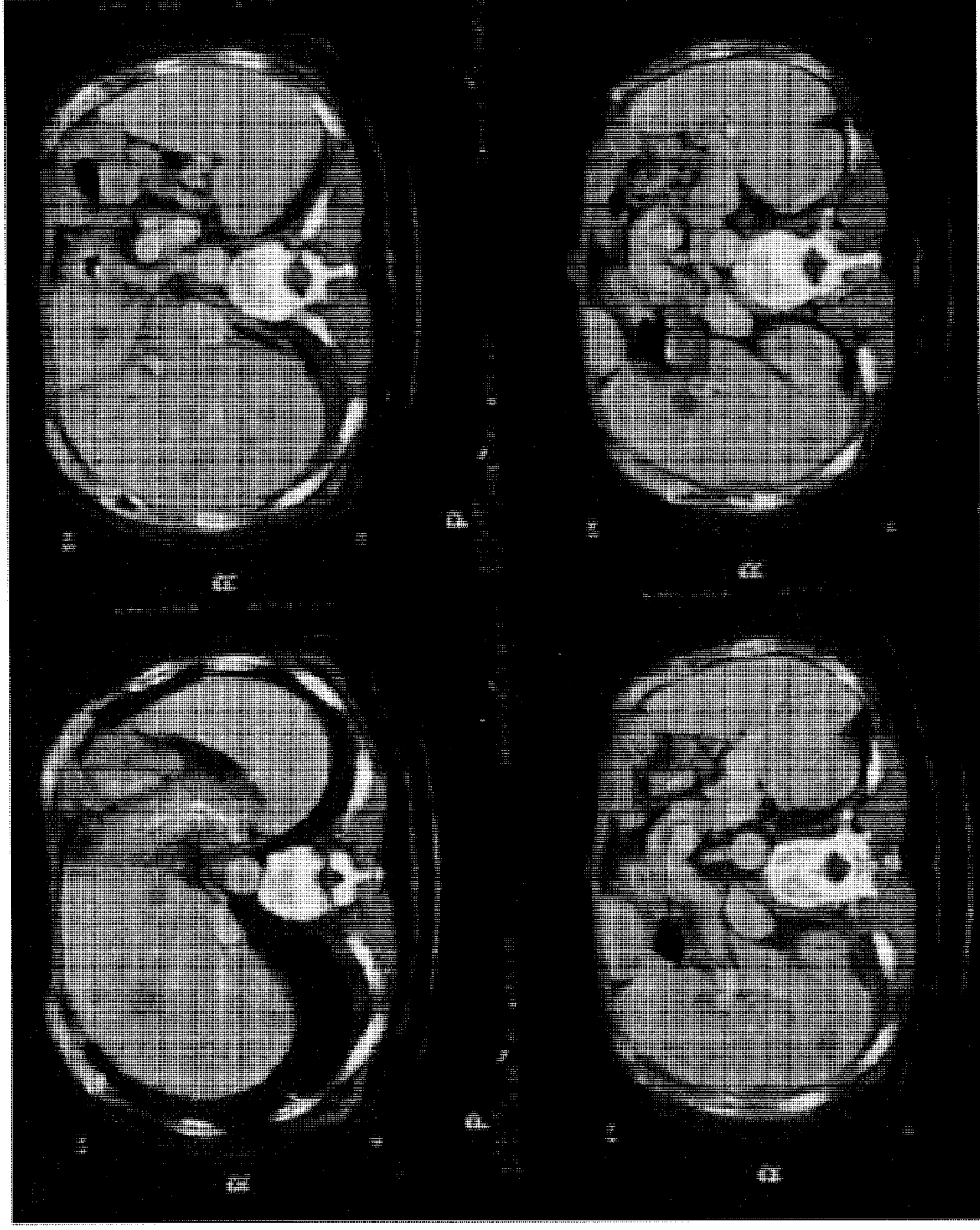
# Cancer Risk following Renal Transplant

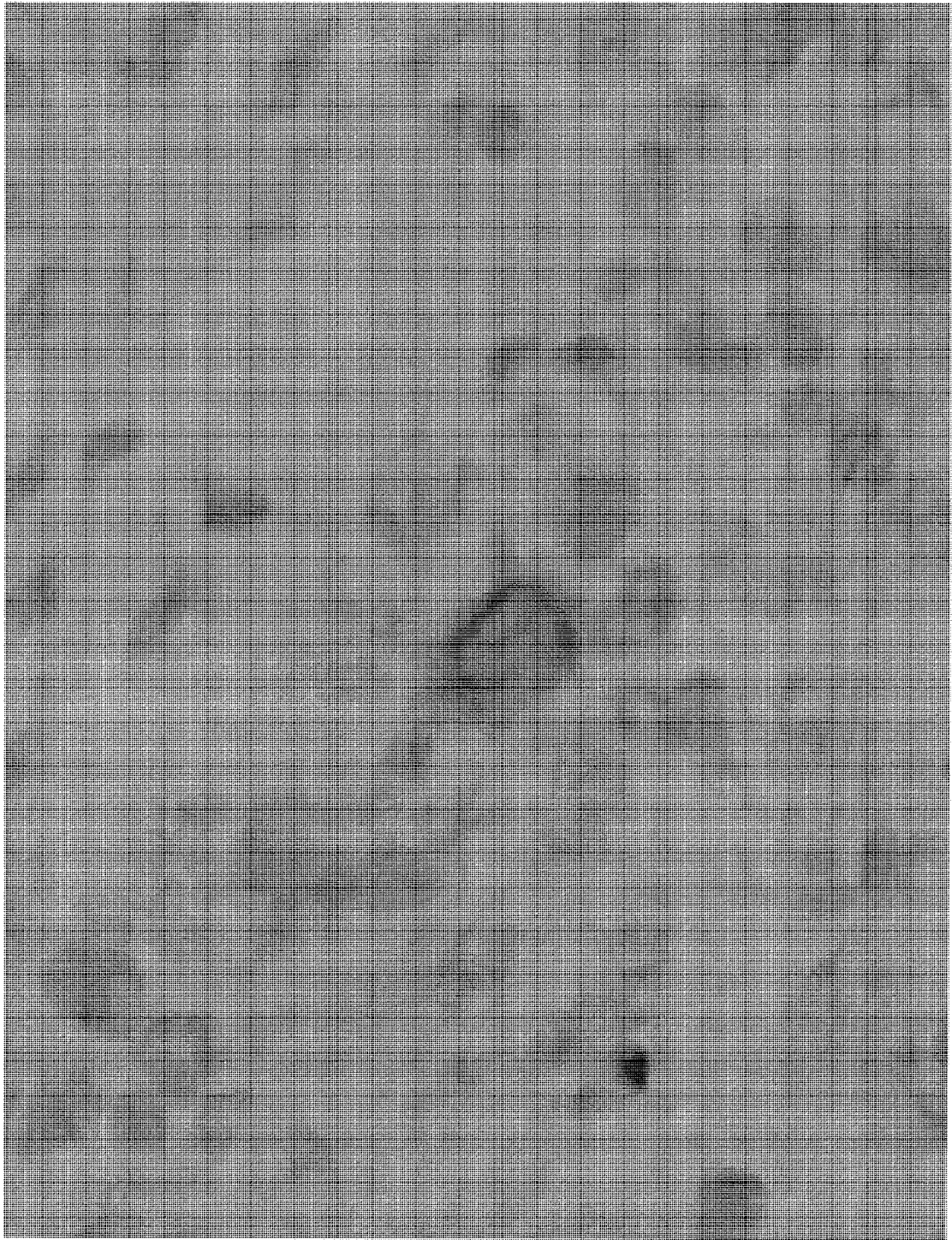
ANZ Dialysis & Transplant register 1997 8618 patients

Cancer	Number	Risk Ratio
CNS Lymphoma	17	>1000
Ureter	10	250
Parathyroid	2	200
Kaposi Sarcoma	18	86
Vulva/Vagina	40	43
Penis	7	24
Cervix	65	17
Bladder	54	7
Kidney	8	7
NH Lymphoma	83	7
Liver	8	6
Colon	50	2
Breast	42	1

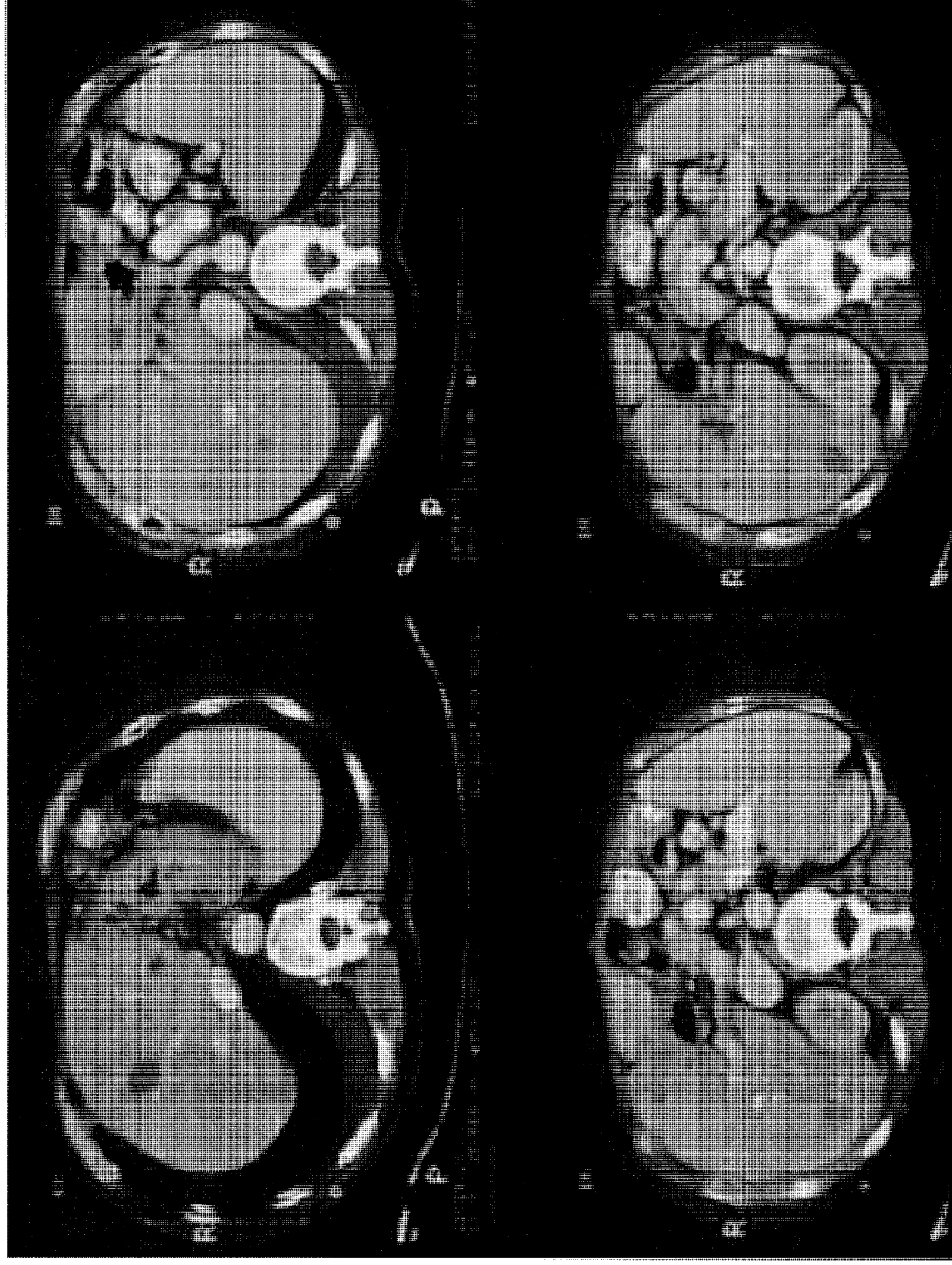


## Lymphoma in a Liver Transplant recipient

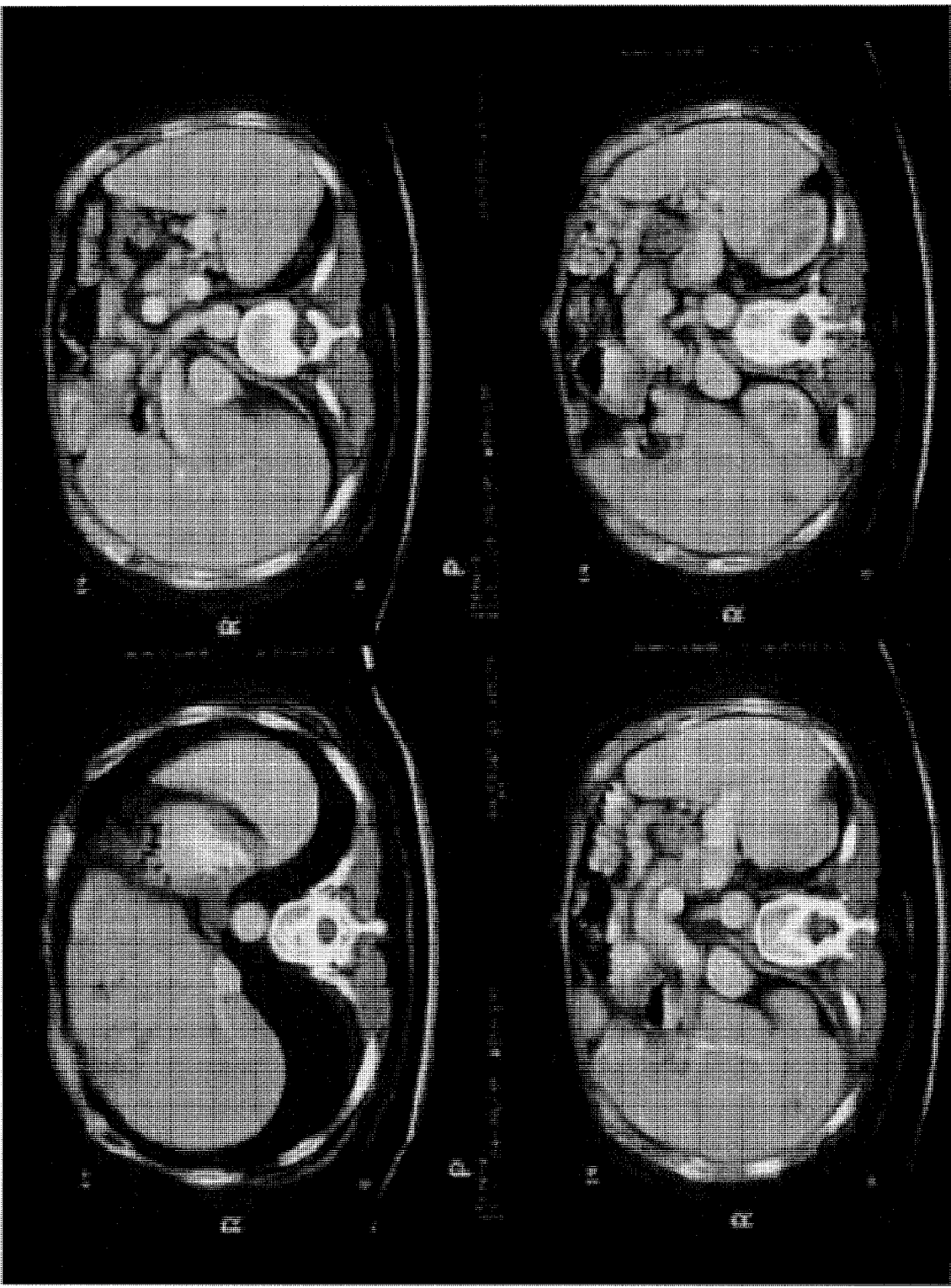




## Post CHOP x3

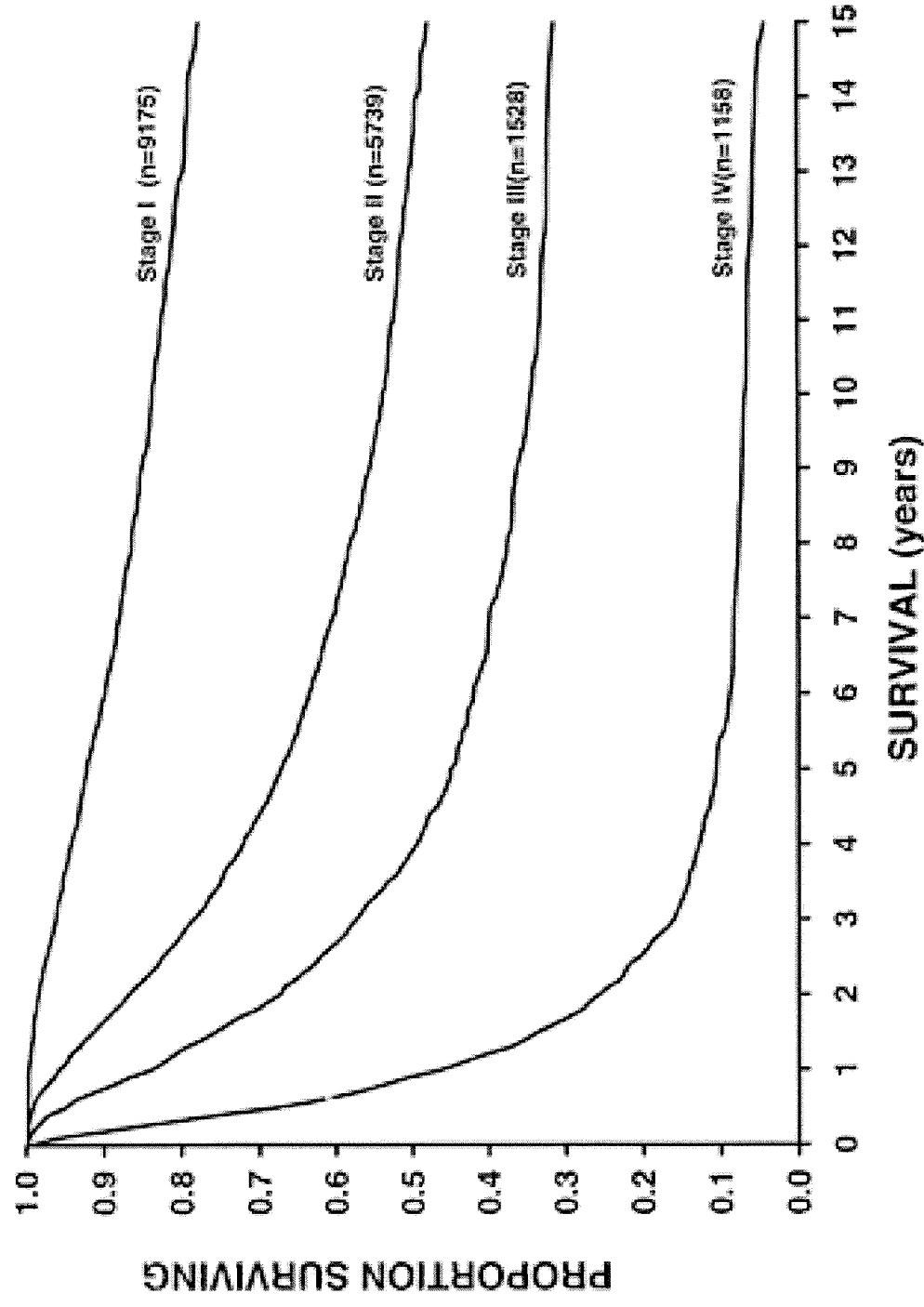


## Off Treatment



Spontaneous immune responses  
can occur in some cancer patients

# Melanoma Stage & Survival



# Malignant Melanoma

- Spontaneous regression observed
- Lymphocyte infiltration of primary tumor is associated with better prognosis
- Responds to immune manipulation
  - non - specific :IFN- $\alpha$ , IL-2
  - Vaccines
- Autoimmune phenomena
  - Vitiligo
  - chorioretinitis
- Antibody responses have been documented eg to gangliosides, NY-ESO-1

# Melanoma associated chorioretinitis

- Level 5 melanoma diagnosed '86
- recurrence lung & lymph nodes excised Sep 90
- multiple cutaneous recurrences
- Chemotherapy
- Night blindness, reduced central vision Aug/91
- Serum: immunofluorescence on unfixed retina (bipolar cells)
- Melanoma - associated chorioretinitis
- Small bowel metastases resected Apr/94
- Currently free of disease

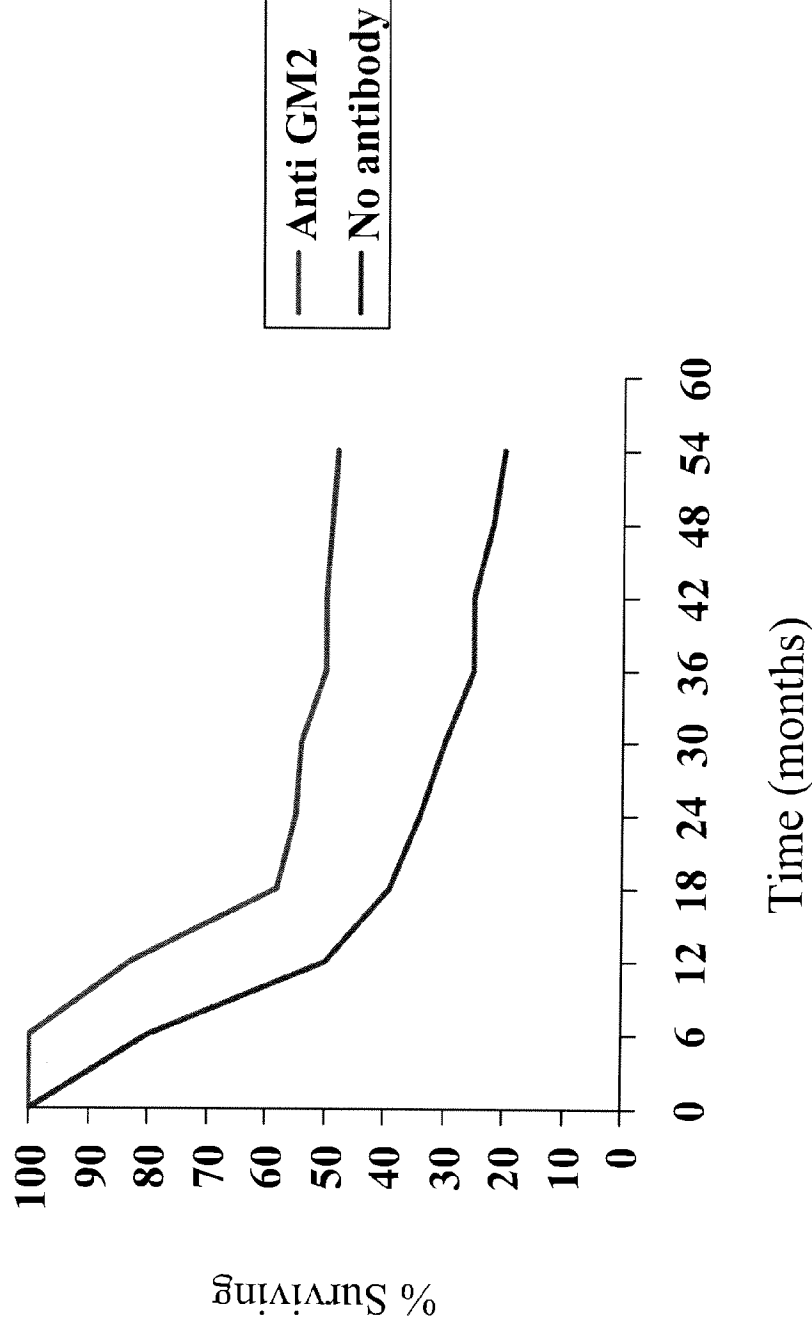


# Antigens identified:

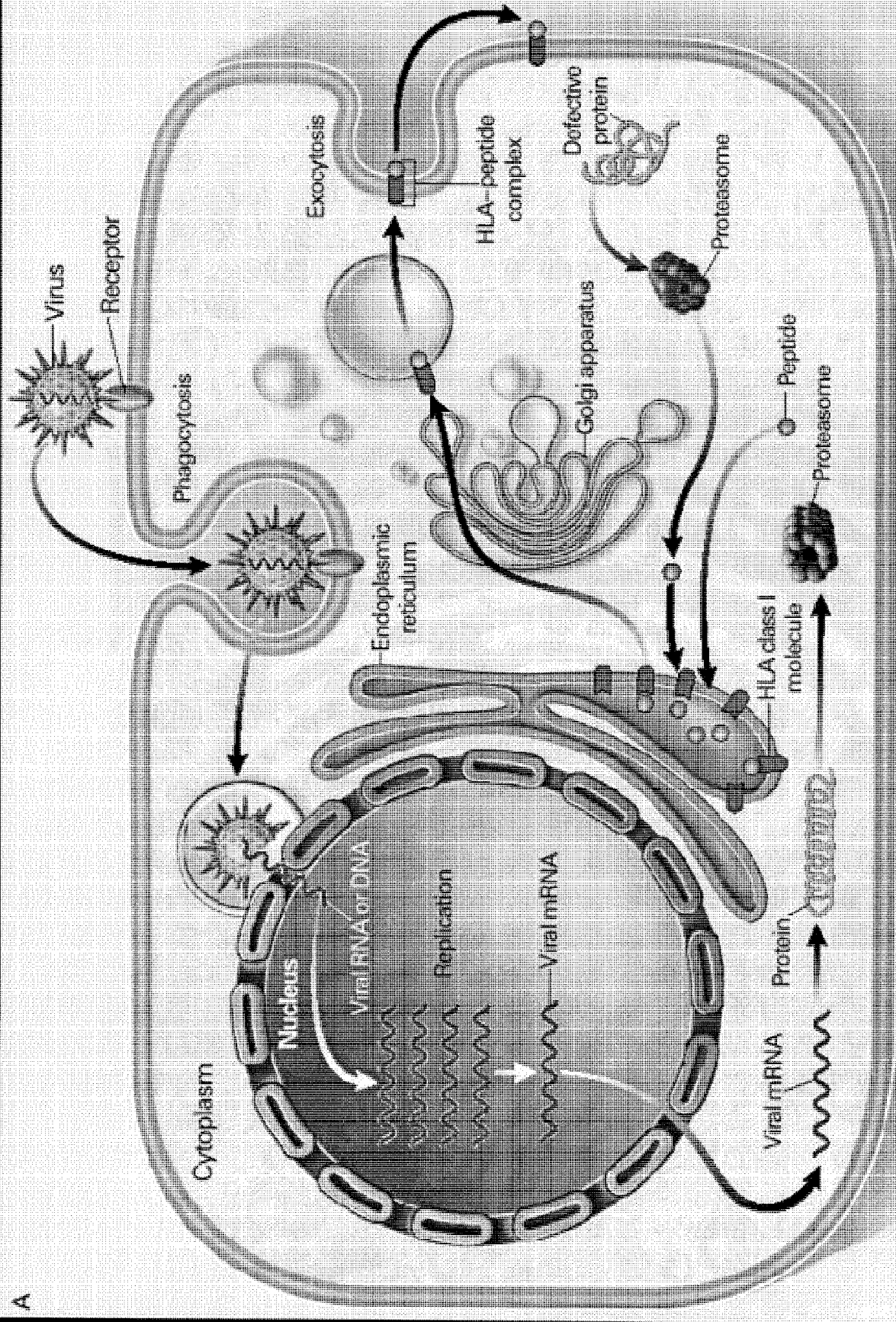
- Cytotoxic lymphocyte targets:
  - Differentiation Ags
    - MelanA/MART-1
    - gp100
    - Tyrosinase
  - CT antigens
    - MAGE family
    - NY-ESO-1
- Antibodies
  - Gangliosides GM2
  - Spontaneous : observed in ~10%
  - associated with improved survival

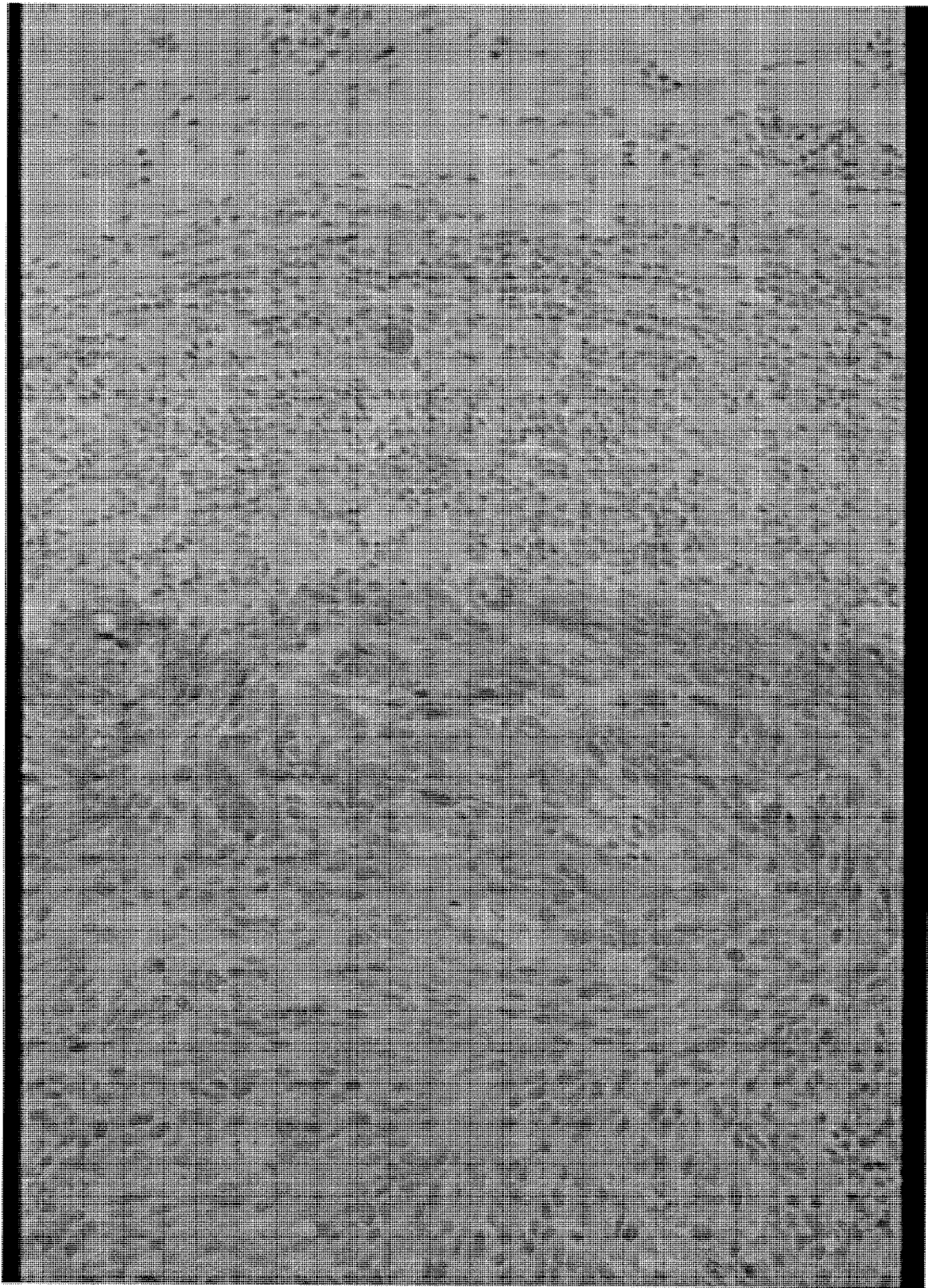
# Improved survival in stage III melanoma patients with GM2 antibodies: a randomized trial of adjuvant vaccination with GM2 ganglioside. Livingston PO, et al

J Clin Oncol 1994 May;12(5):1036-1044



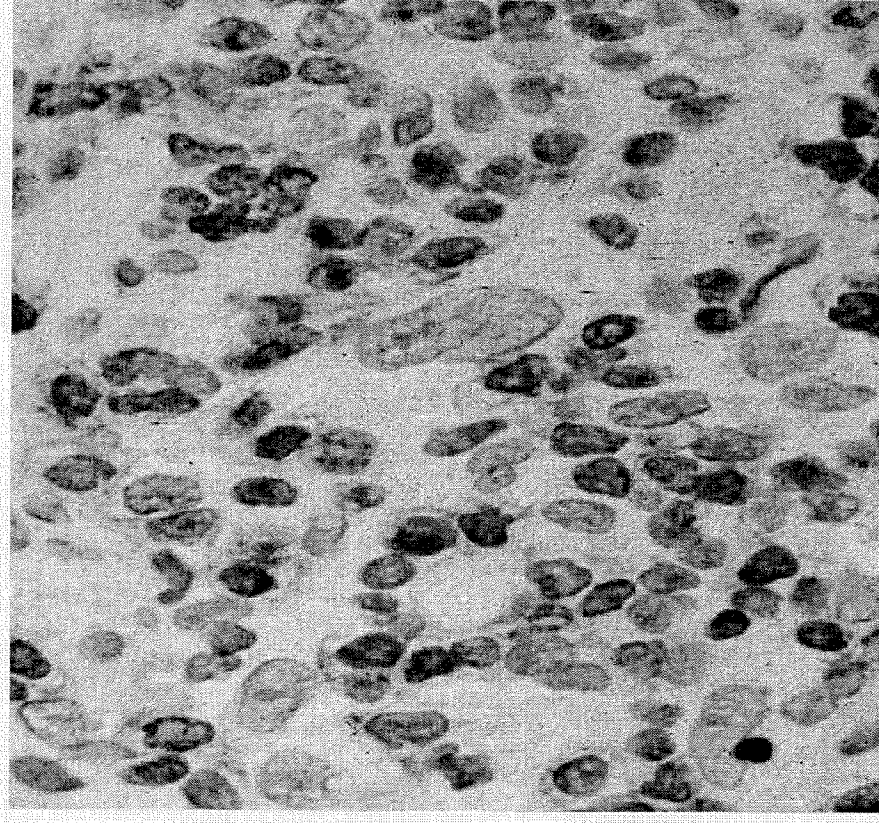
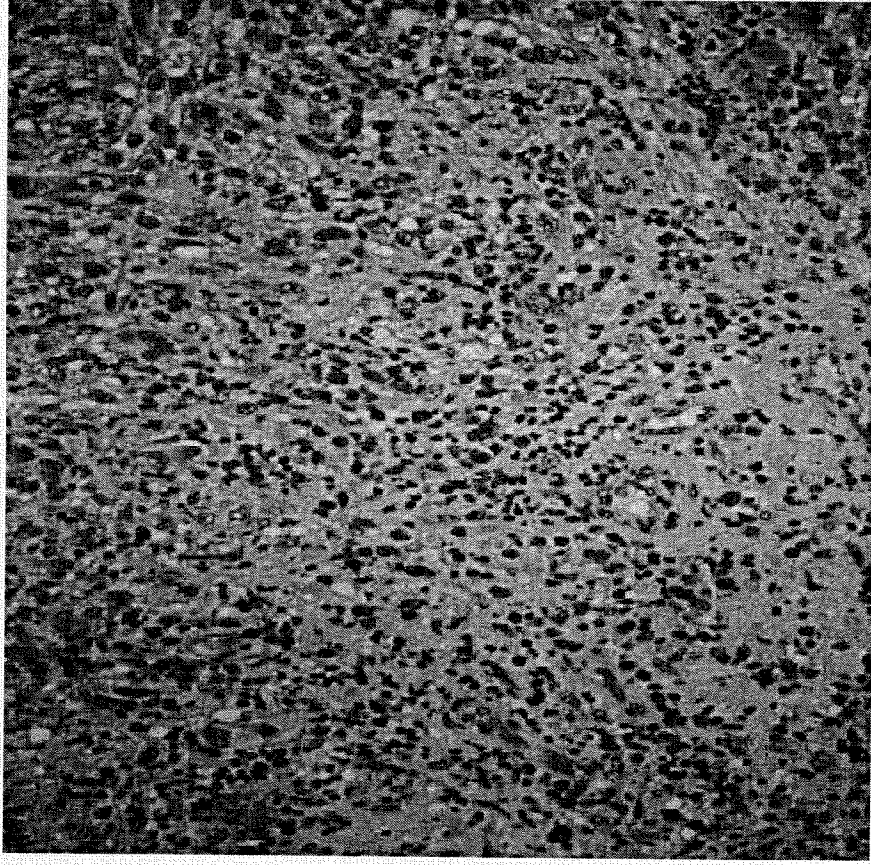
A





Immune responses to tumour  
antigens may affect clinical  
outcomes in some patients



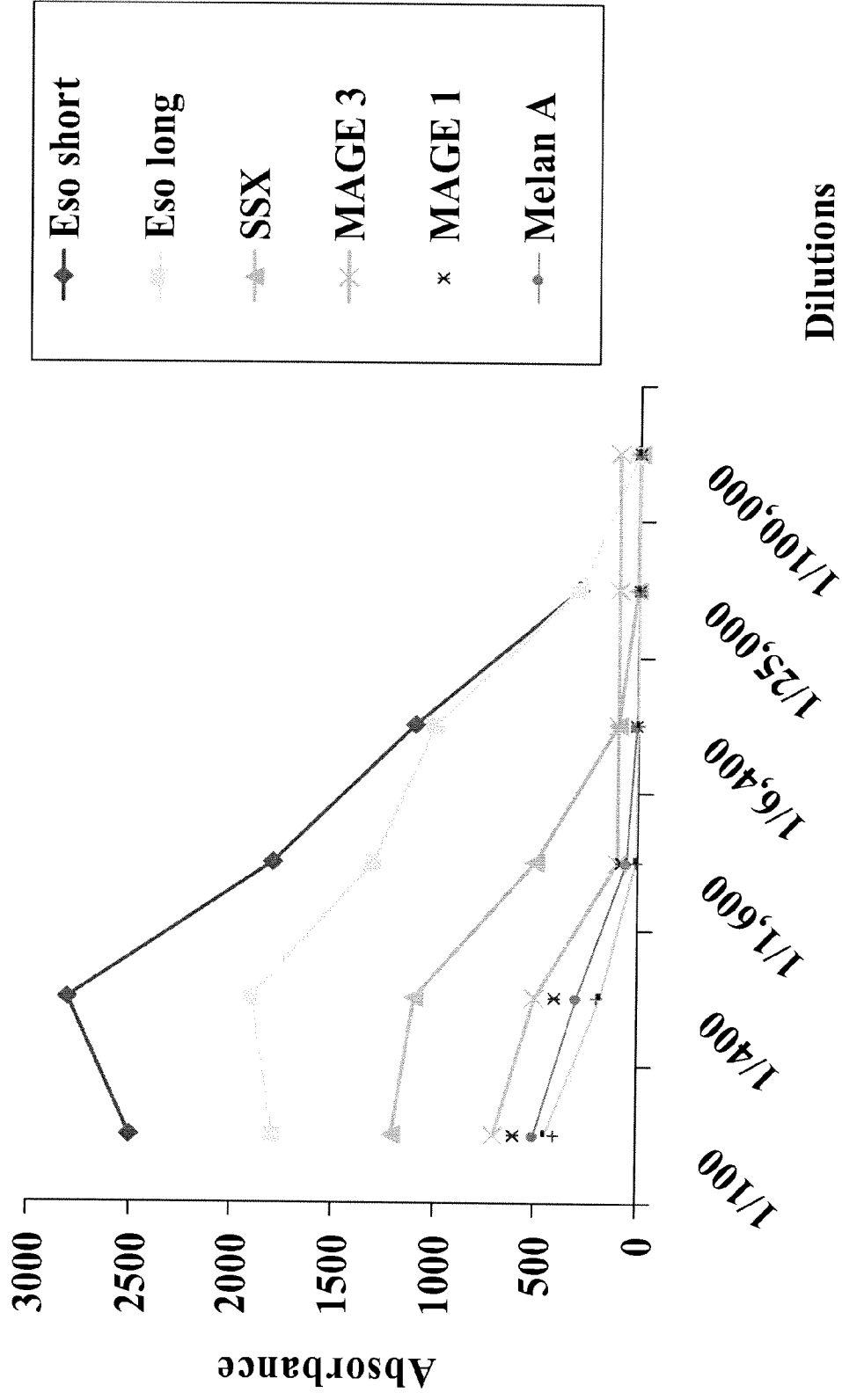


Neutrophilic Infiltrates with Oncofibrinocytes

# Indolent Melanoma

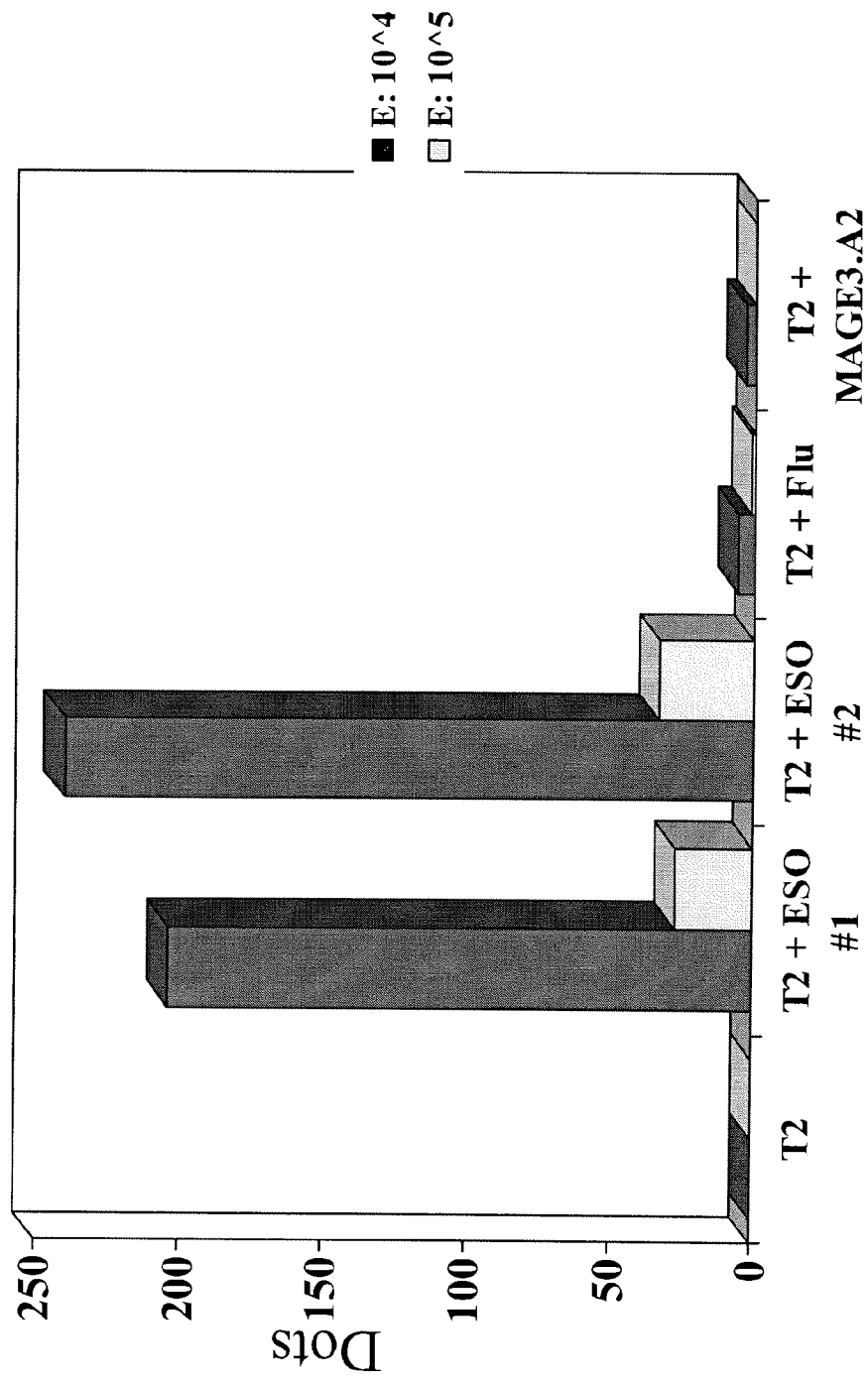
- Initial diagnosis: Dec 87
- Adjuvant BCG
- Relapse in iliac and retroperitoneal lymph nodes:  
Nov 93
- Vitiligo
- slowly progressive - treated with chemotherapy  
and radiotherapy
- Extensive necrosis of tumor on CT scan
- plasma cell infiltrate in tumor
- Died Apr 97

# Indolent Melanoma: ELISA for Tumor Ags



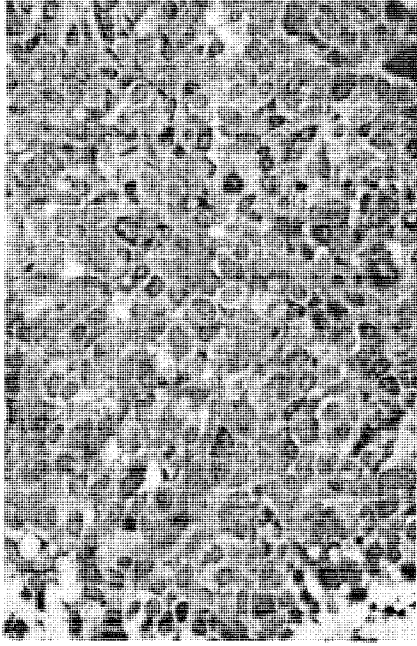


# ELIspot assay: NY-ESO-1



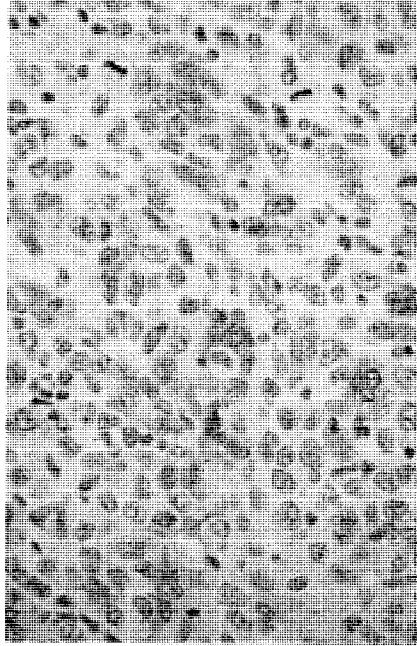
# NY-ESO-1

CT 'Cancer testis' antigen	Highly immunogenic
Cytoplasmic	Epitopes restricted by Class I
Unknown function	•HLA A2
180 amino acids	•HLA Cw3, Cw6
Expressed in testis, trophoblast	Class II
Variety of cancers	•HLA DP4
•Melanoma	•DR53
•Hepatocellular Carcinoma	•DR4
•Lung	Heterogenous expression
•Bladder	•RT-PCR
•H&N	•Antibody (ES121, E978)
•Synovial sarcoma	
•Breast	



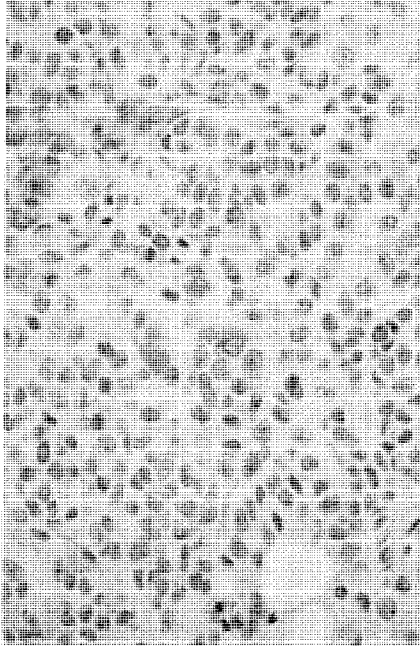
### **NY-ESO-1 Rich**

IHC >50% cells staining, ++ or greater  
20/120 melanomas **(18%)**  
PCR +ve: 20/20 (100%)



### **NY-ESO-1 Intermediate**

20/120 Melanomas **(18%)**  
PCR +ve: 15/20 (75%)



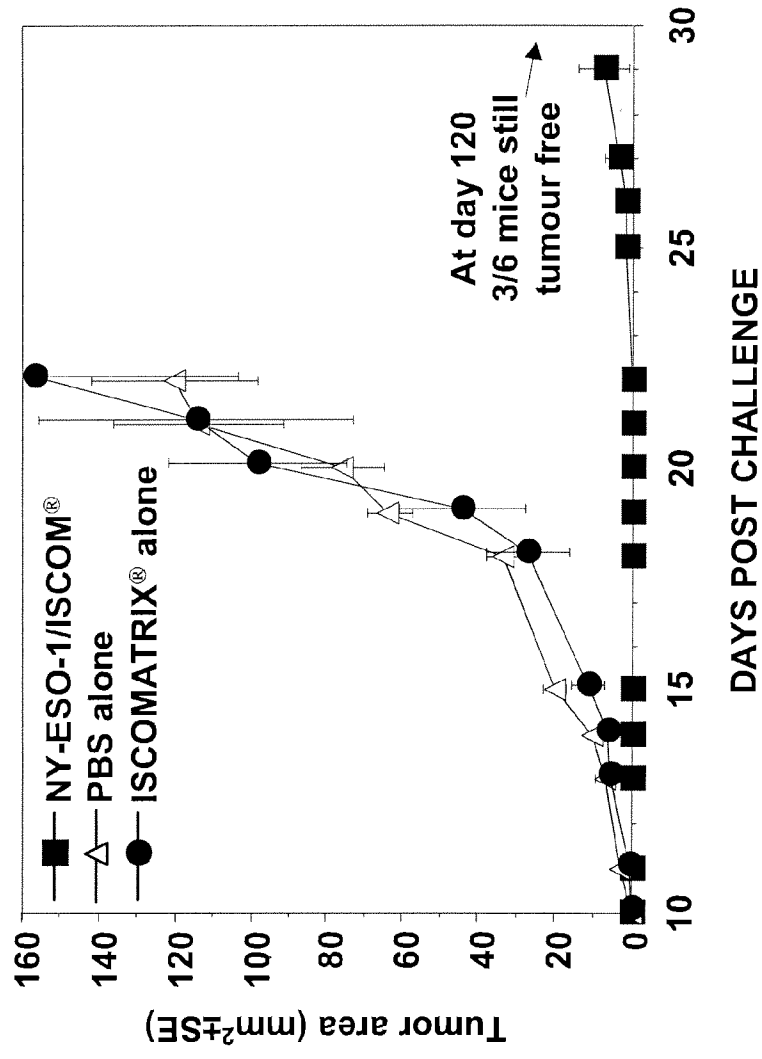
### **NY-ESO-1 Poor**

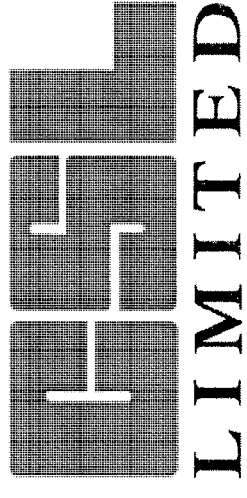
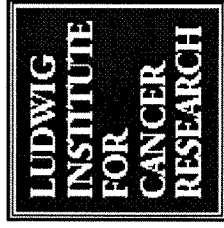
<25% + or <5% any intensity  
6/120 melanomas **(5%)**  
PCR +ve: 3/6 (50%)

### **NY-ESO-1 Negative**

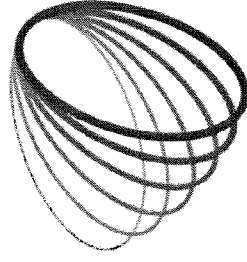
66/120 melanomas **(59%)**  
PCR +ve: 14/66 (21%)

Figure 8. Maraskovsky et al.,



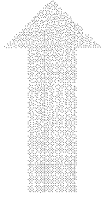


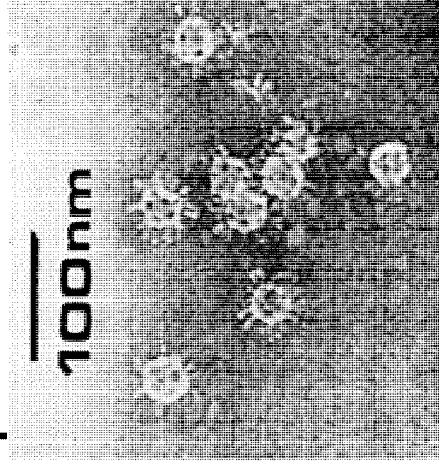
**A phase I study of NY-ESO-1 ISCOM<sup>®</sup>  
in patients with NY-ESO-1 positive  
cancers and minimal residual disease**



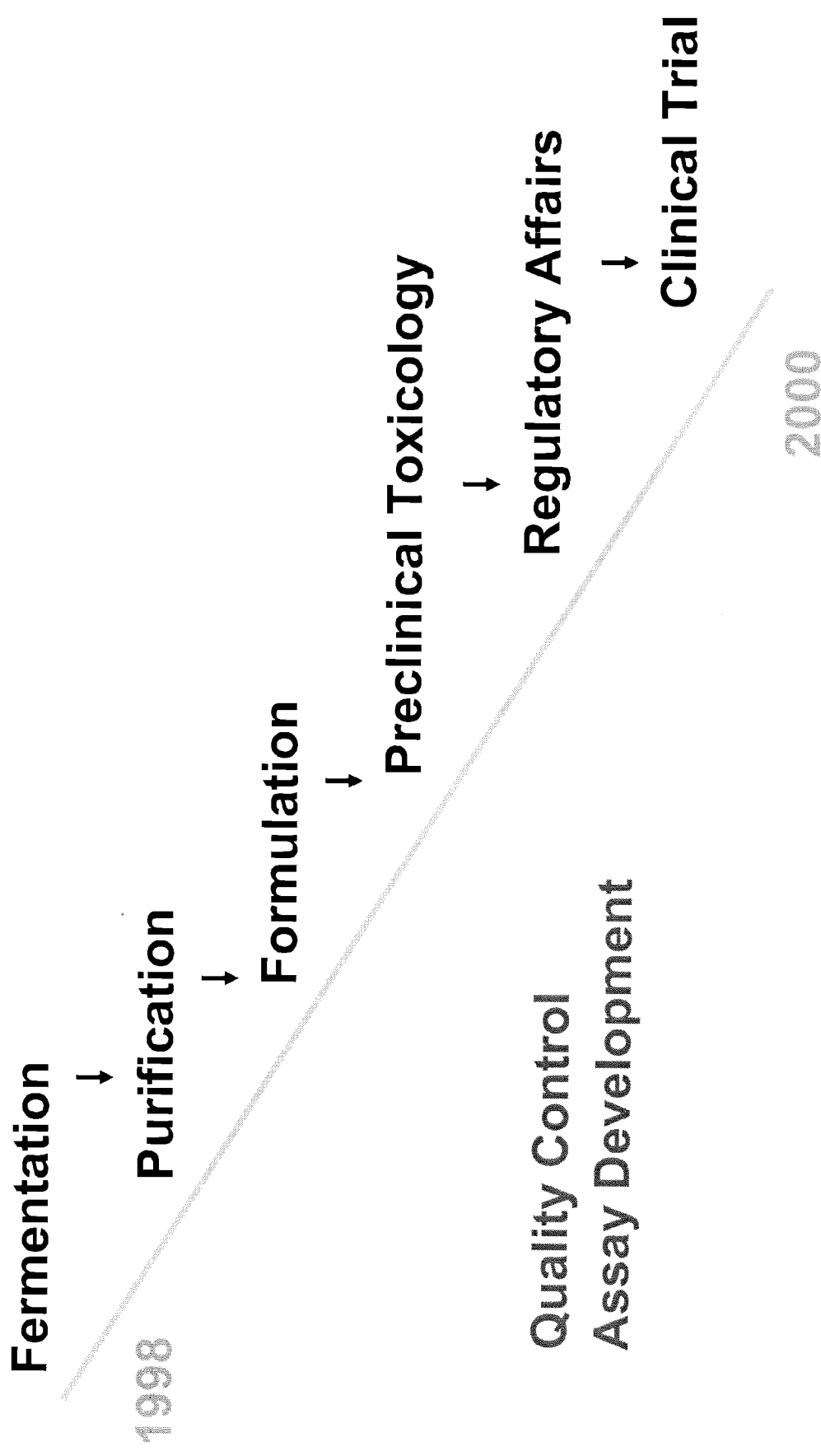
**CDCT**

# ISCOM®

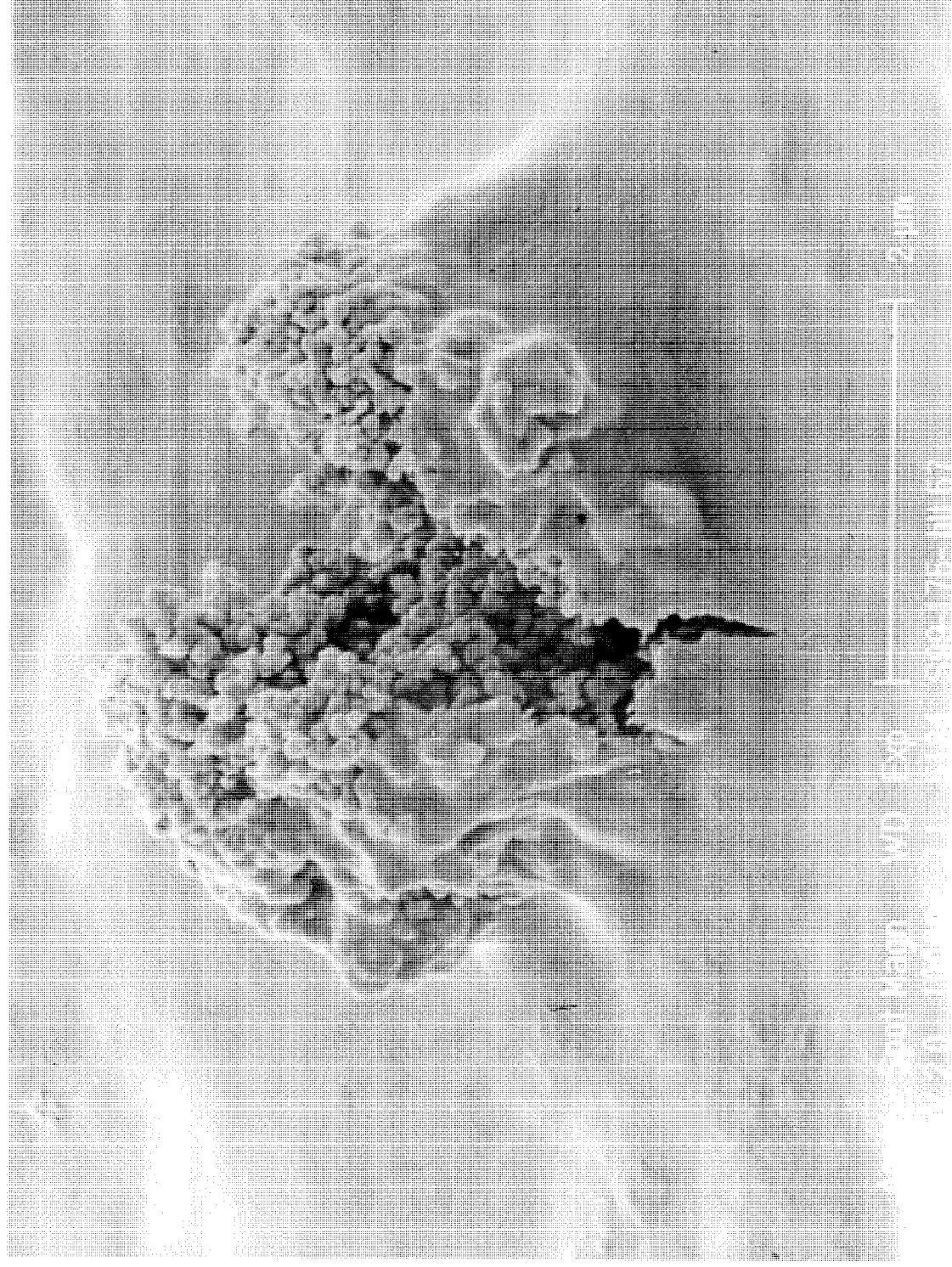
- Adjuvants
  - humoral  Aluminium salts
  - cellular ?
- Immuno Stimulating Complexes
  - ISCOM™
  - ISCOMATRIX™



# Vaccine Production Timeline

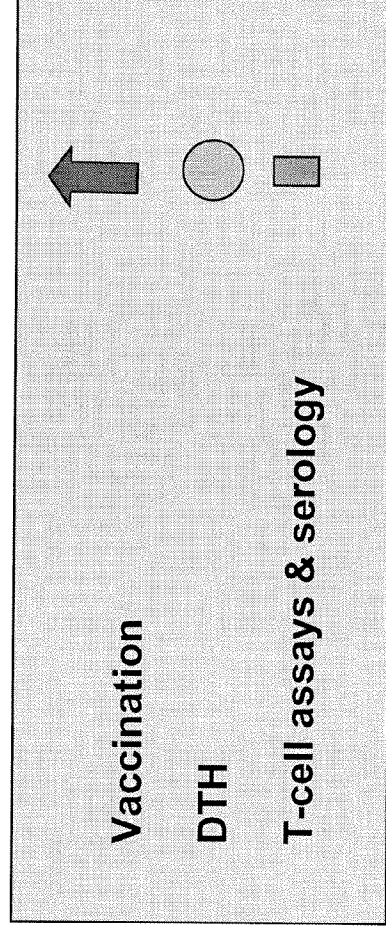
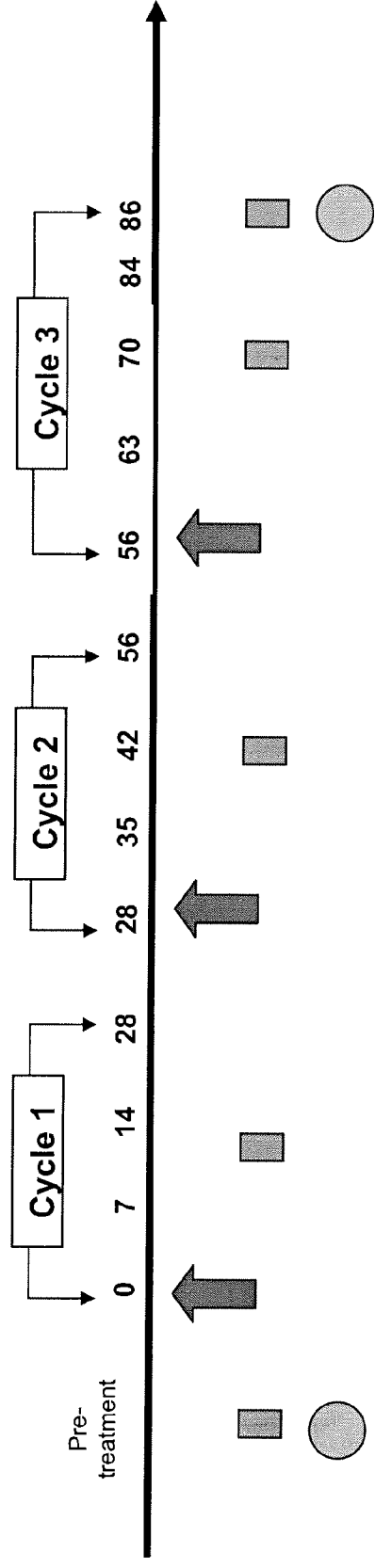


# Scanning EM of NY-ESO-1 ISCOM®





# Study Design



# Patients

- Total 46
- 3 parts
  - 1 NY-ESO-1/ISCOM<sup>®</sup>
    - 3 pts/cohort
    - Dose levels A 10ug & B 30ug
    - Only HLA A2+ patients for purposes of immunological assays
  - 2 NY-ESO-1/ISCOM<sup>®</sup> - dose level C
    - Dose 100ug expanded to 20 patients
    - 10 HLA A2+ve (2 placebo), 10 HLA A2-ve (2 placebo)
  - 3 Protein alone - dose level D
    - 100ug expanded to 20 patients
    - 10 HLA A2+ve (2 placebo), 10 HLA A2-ve (2 placebo)

# Cancer Types

<b>On Study</b>	<b>51</b>
<b>Melanoma*</b>	<b>46</b>
<b>Ca Breast</b>	<b>3</b>
<b>TCC Bladder</b>	<b>1</b>
<b>Adenoid cystic carcinoma</b>	<b>1</b>

\*Stage II, III and IV resected

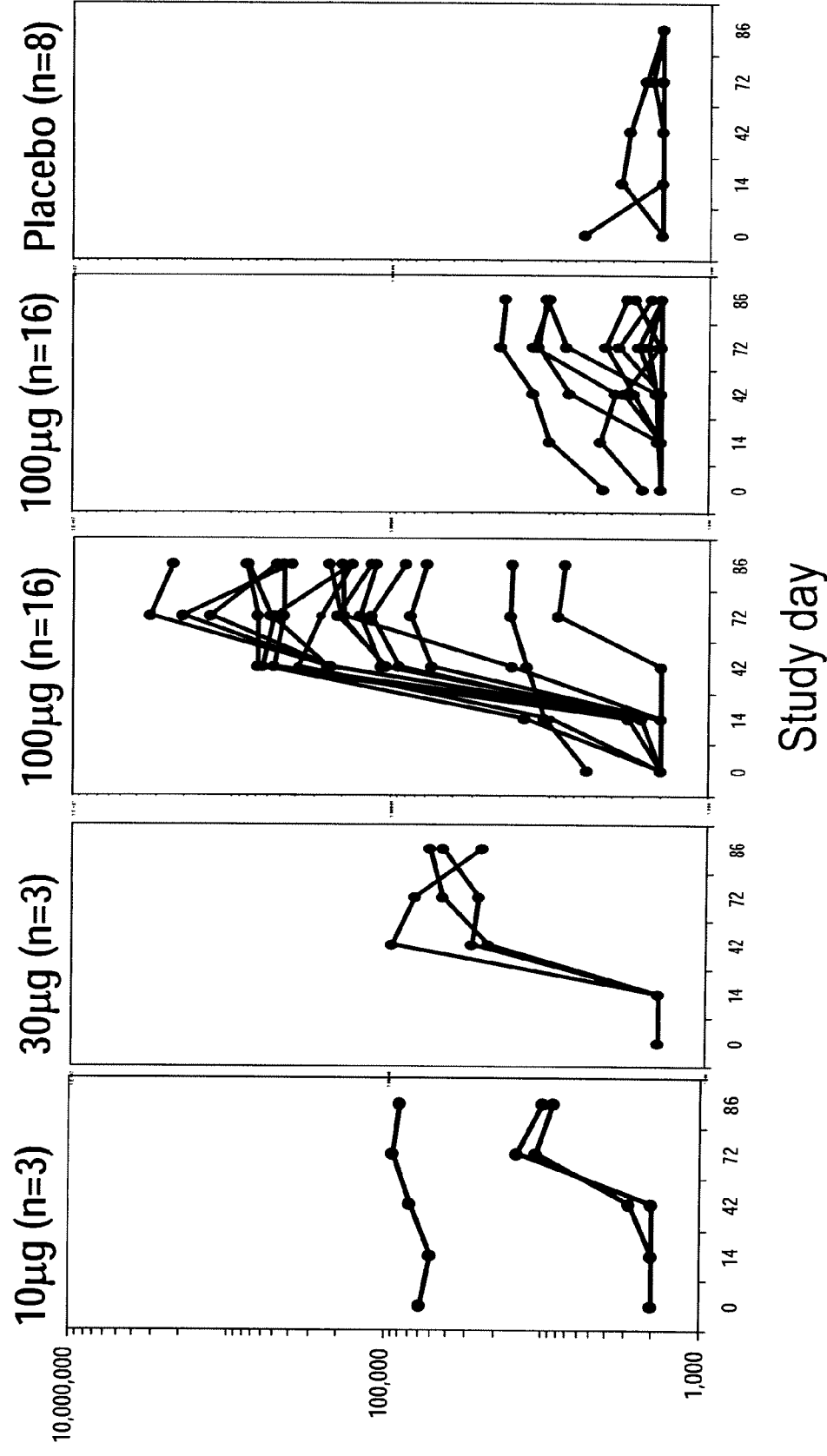
# Toxicity

- NY-ESO-1 ISCOM<sup>®</sup> was well tolerated
- Most adverse events were grade 1 or 2
- Grade 3 toxicities: injection site pain in 3/46
- Common grade 2 toxicities (2 or more patients)
  - Injection site pain
  - Fever
  - Myalgia
  - Headache
  - Flu-like symptoms

# Assays

- DTH using NY-ESO-1 protein alone
- Antibody (capture ELISA)
- CD8+ T cells
  - Tetramer: SLLMWITQC
  - Cytospot:  $\gamma$ IFN producing CD8+T Cells)
- Assays under development
  - CD4+ T cells (*DC & protein: cytokine secretion*)
  - Class I epitopes - non HLA-A2

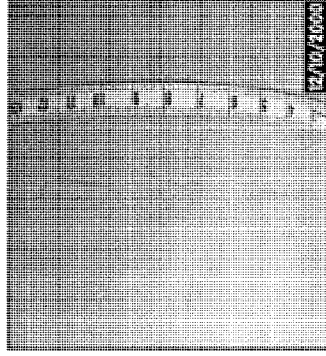
# Antibody titre by cohort



# Delayed-type Hypersensitivity: 1 µg protein

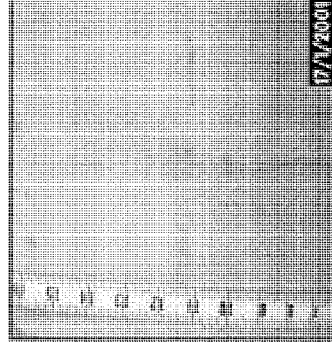
Dose B (A2+) : 30µg  
NY-ESO-1-ISCOM®

105/J-M



PRE

Erythema = 13  
Induration = 14

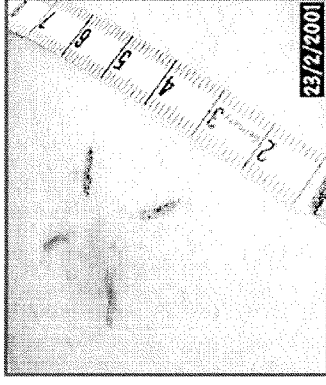


Day 86

Erythema = 60  
Induration = 12

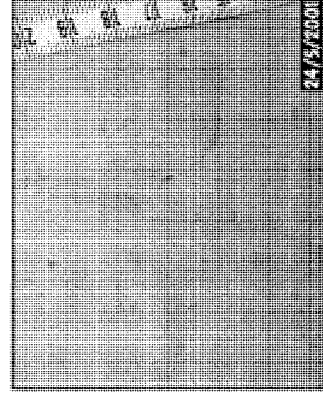
Dose C (A2-) : 100µg  
NY-ESO-1-ISCOM® /  
Placebo

115/N-F



PRE

Erythema = 25  
Induration = 4

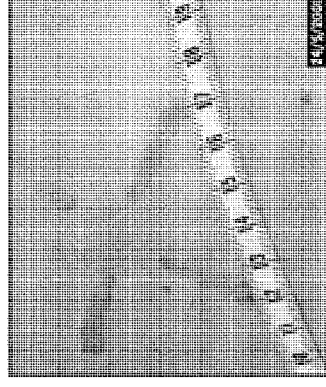


Day 86

Erythema = 50  
Induration = 34

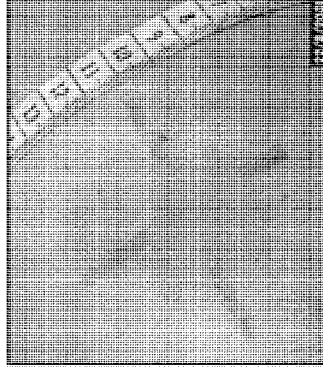
Dose C (A2+) : 100µg  
NY-ESO-1-ISCOM® /  
Placebo

126/KLE



PRE

Erythema = 15  
Induration = 3

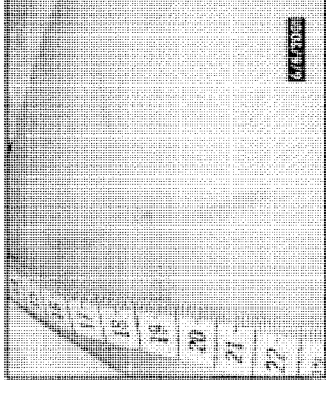


Day 86

Erythema = 60  
Induration = 25

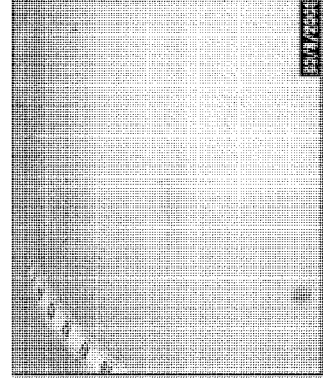
Dose D (A2-) : 100µg  
NY-ESO-1 Protein /  
Placebo

127/JSM



PRE

Erythema = 2  
Induration = 0

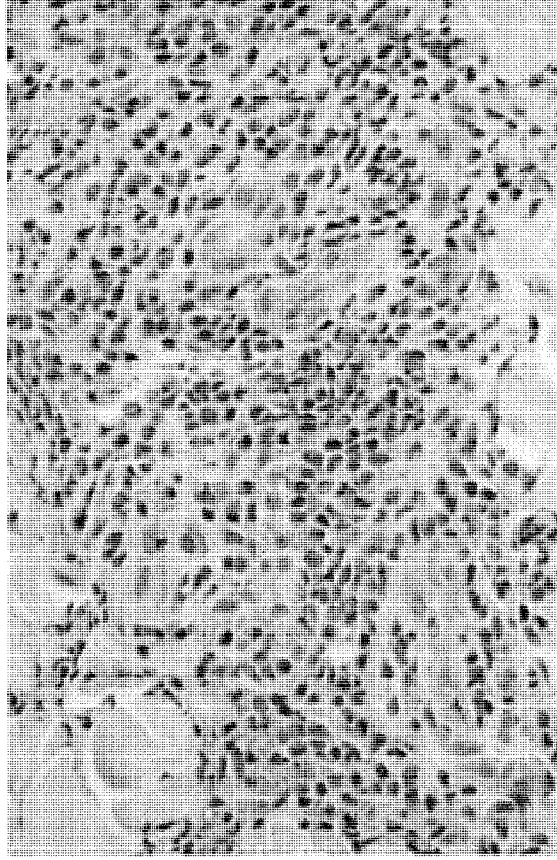


Day 86

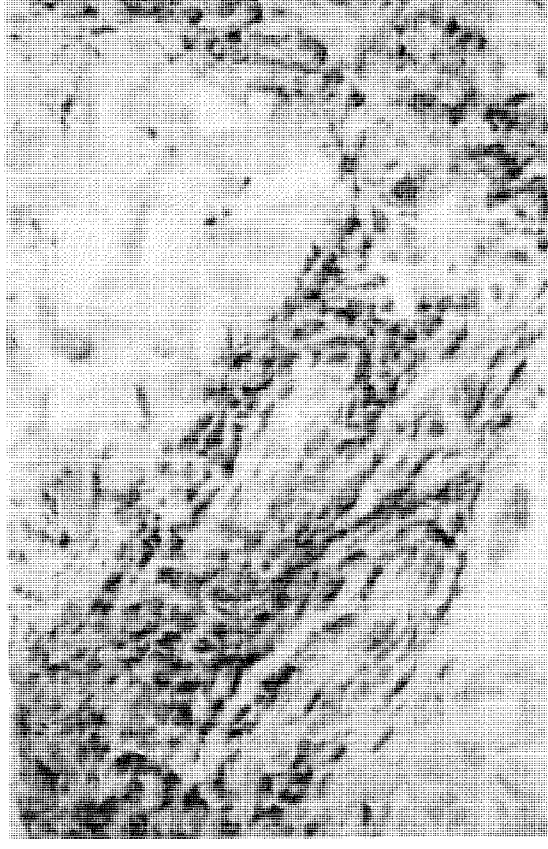
Erythema = 12  
Induration = 0

# DTH response to 1mg NY-ESO-1 protein

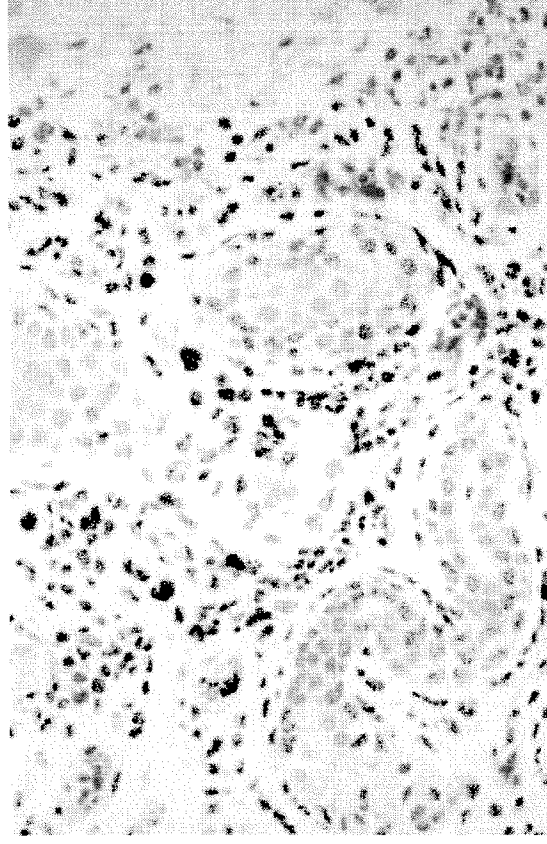
**H&E**



**CD4**

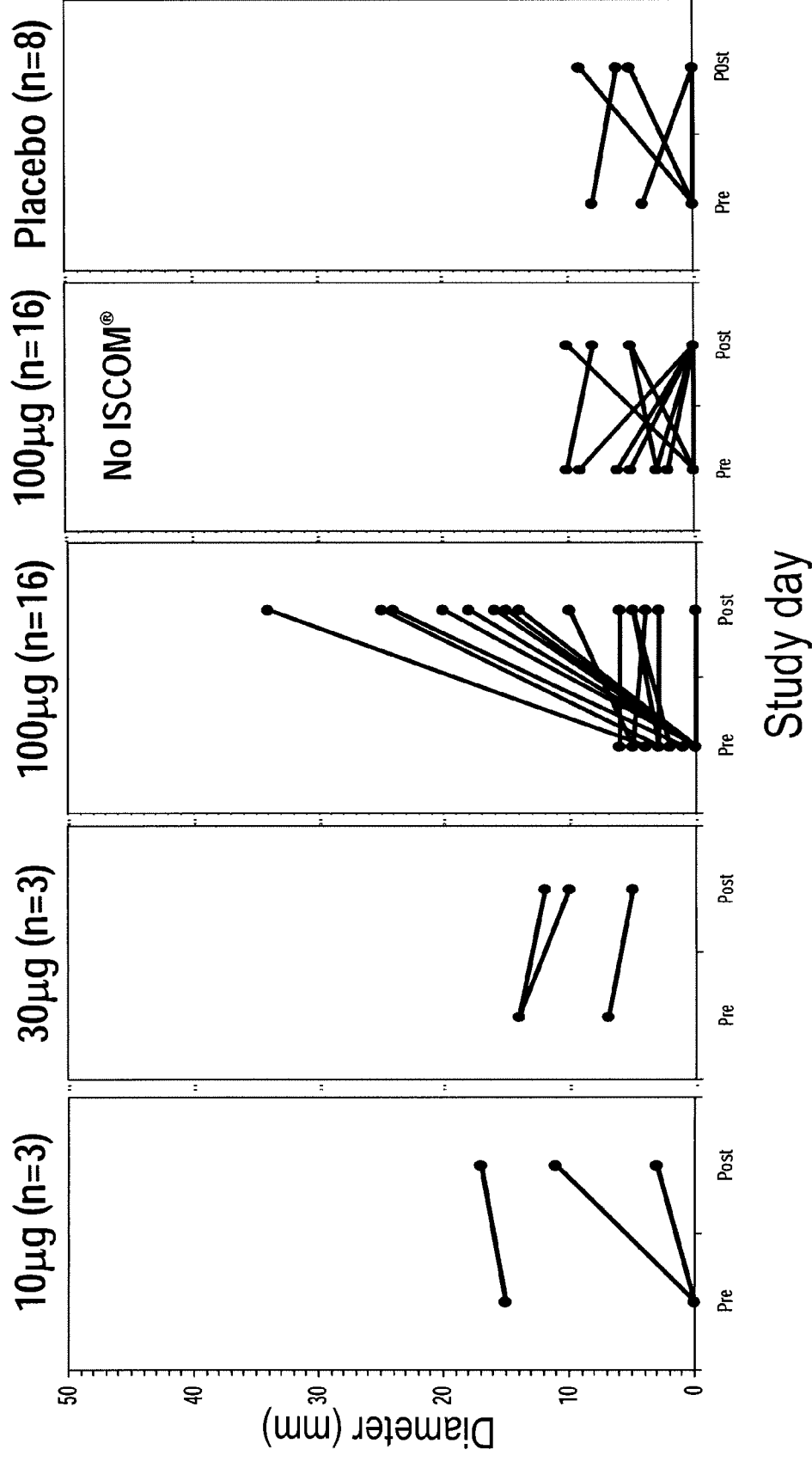


**CD8**





# DTH Induration by cohort

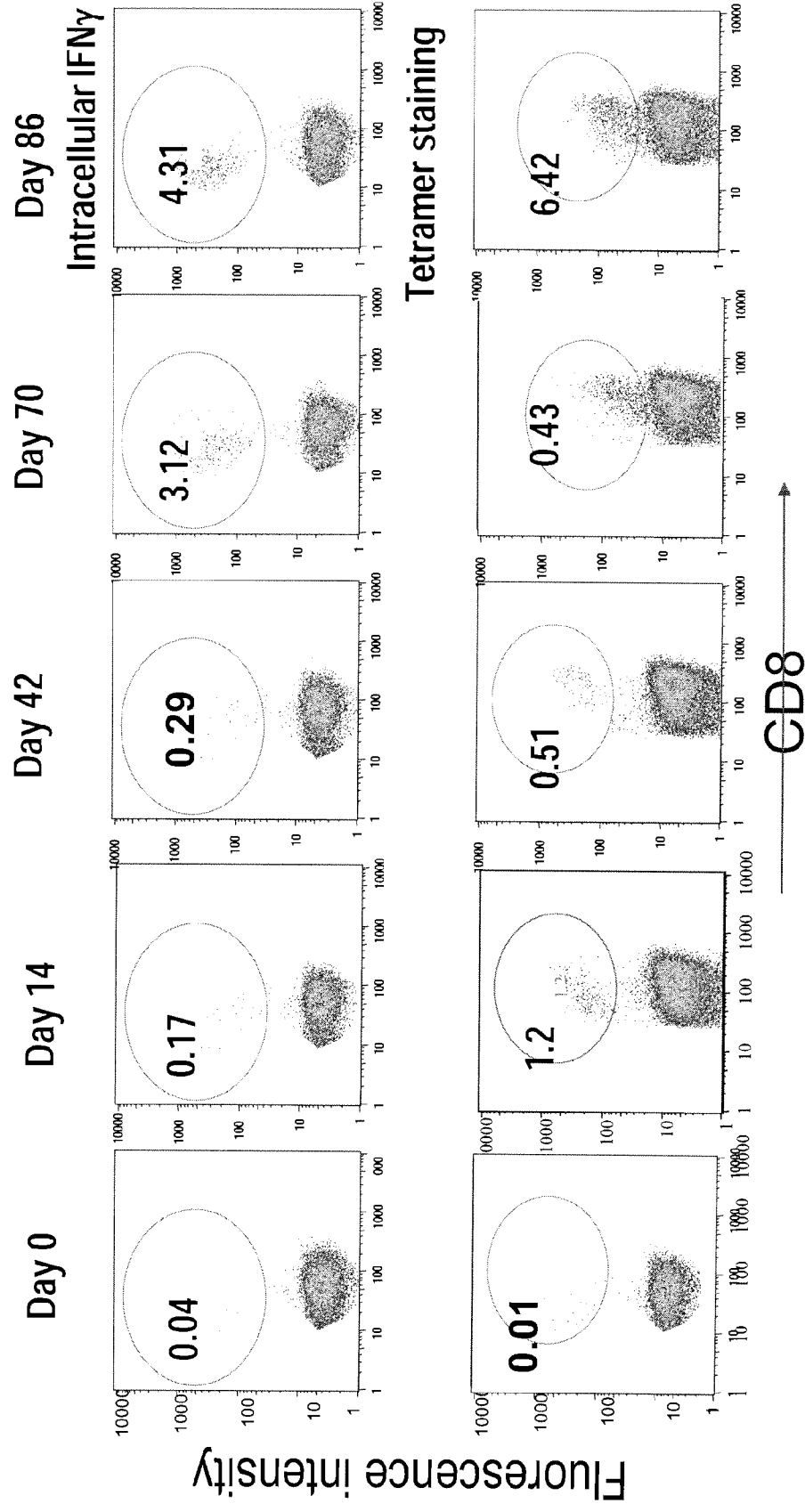


# Cytospot assay method

- Blood collected, ficoll and frozen
- Batch assayed on one day
- PBMC activated with peptide in bulk culture using peptides (0.5mM) in the presence of 250mM TCEP (a reducing agent for breaking the disulfide bonds formed between ESO peptides).
- An internal control was established to enable data comparison for multiple time points---EBV BMLF1.280-288-specific CTL
- Cells were expanded for 7 days
- The CTL were activated or T2 cells pulsed +/- peptides
- BFA was directly added and the assay was harvested at 4 hours.
- Cytospot + Tetramer analyses were performed
- Controls:
  - +ve control for NY-ESO-1: Patient with known ESO1a and ESO1b response
  - Control for non-specific immune activation: EBV
  - Control for non-specific activation by T2 cells: Non-pulsed T2 cells
  - Control for non-specific peptide: MAGE 3

# T- cell response: $\gamma$ IFN production

## HLA A2+ pt (peptide SLLMWITQC)



# Summary Immunological Data

DTH (doubling or greater of induration)

A	B	C	D	Placebo
1/3	0/3	11/16	2/16	2/8

Antibody

A	B	C	D	Placebo
3/3	3/3	16/16	4/16	0/8

Cytospot & Tetramer

A	B	C	D	Placebo
1/3	0/3	3/8	1/8	0/4

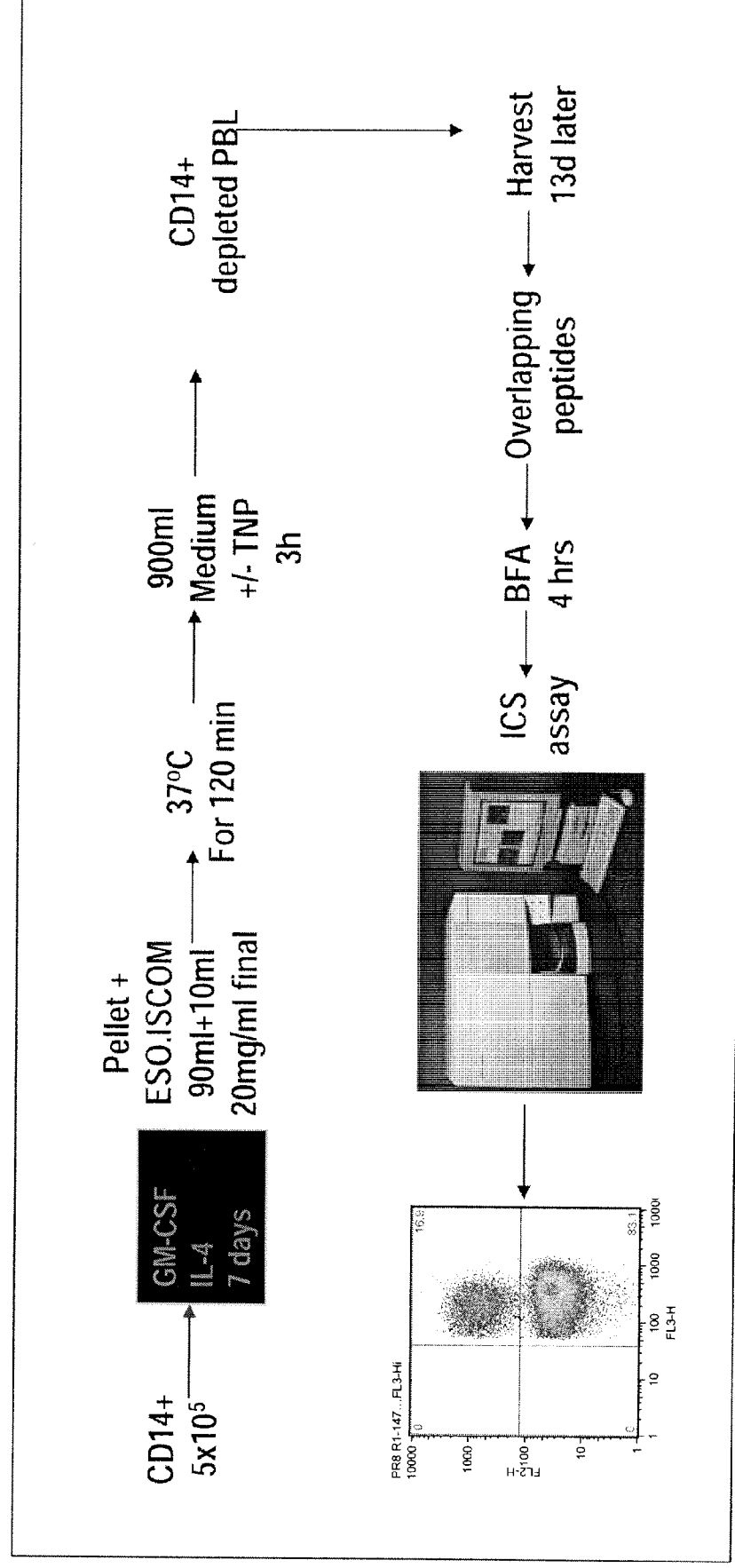
# Conclusions

- NY-ESO-1 ISCOMs vaccinations were safely tolerated
- NY-ESO-1 ISCOMs generated both humoral & cellular responses
- ISCOM adjuvant generated superior DTH and antibody responses
- Cytospot assay in HLA A2+ve patients: positive in 1 level A pt (with prior Ab response), 3/8 level C patients and 1/8 level D patients.
- These responses were seen in patients with and without pre-existing antibody titres
- There was a good correlation between tetramer & cytospot data
- Is there evidence of immune response to other epitopes?

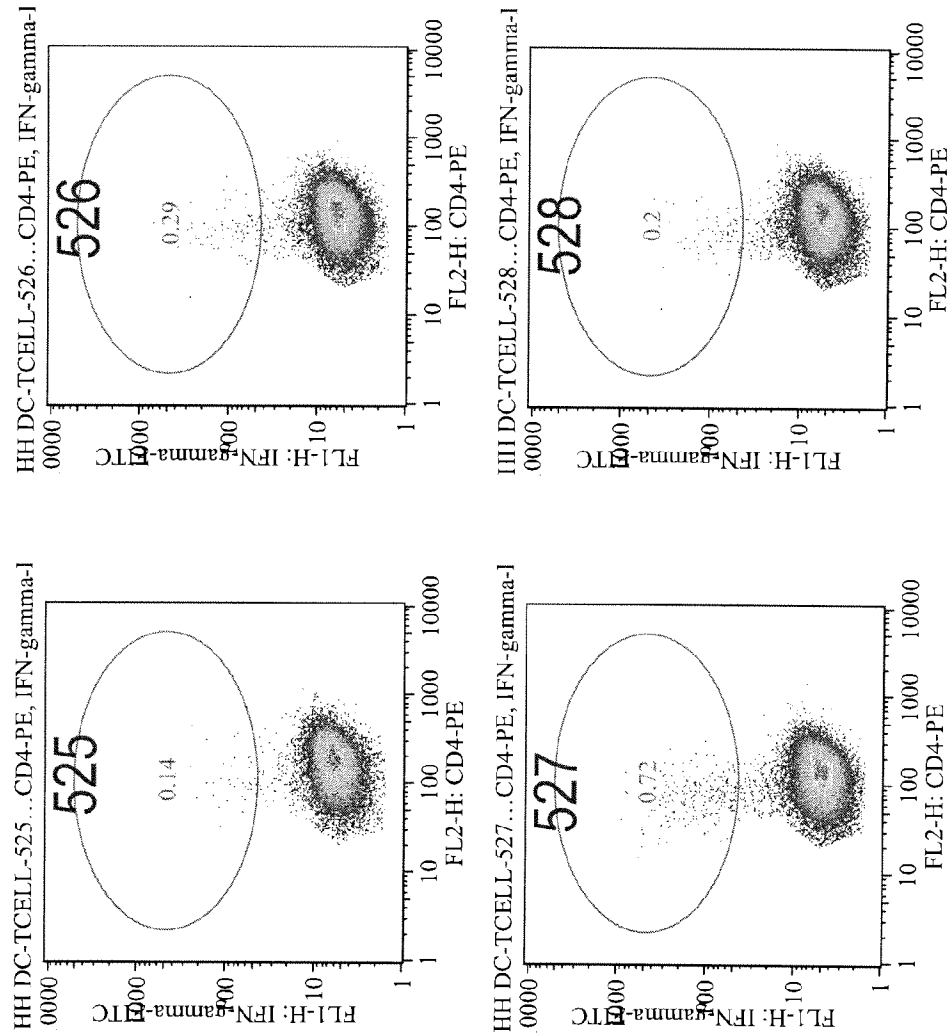
# Identification of response to other epitopes

## Class I & Class II

# Generation of NY-ESO-1 Specific T cells Using Tumor-Ag-loaded Autologous-DC

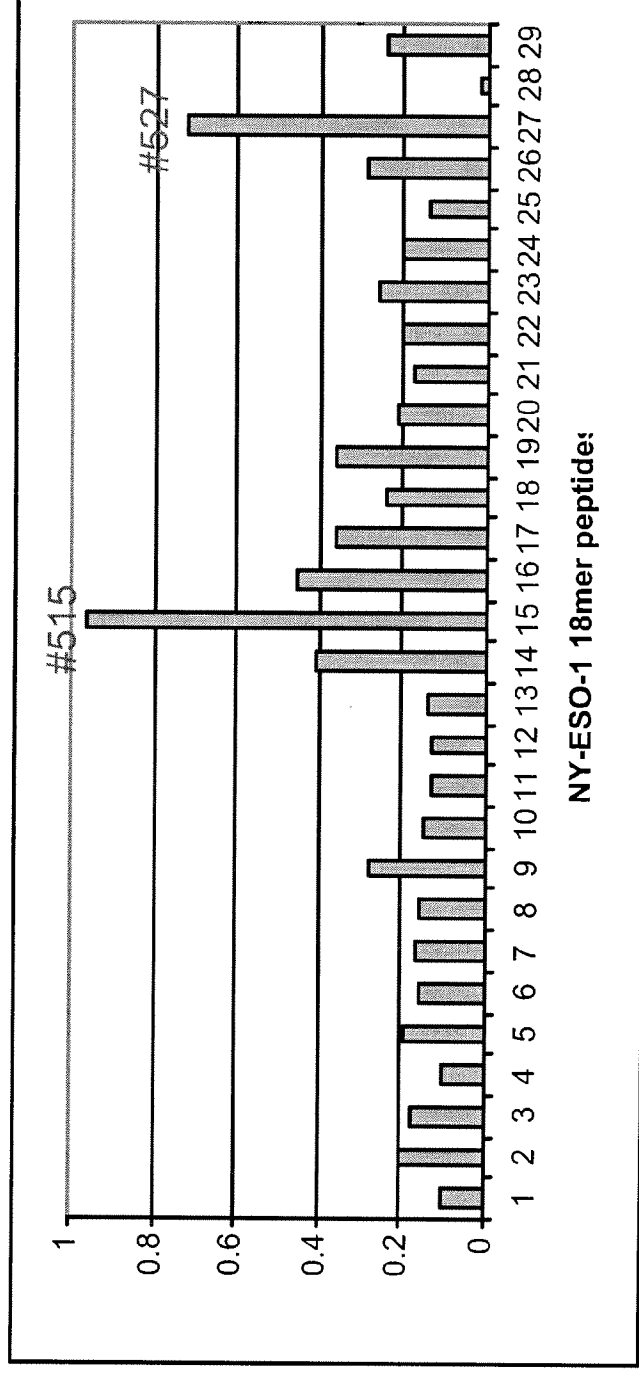


Pt 107 T cells generated with DC+ISCOM/NY-ESO-1 and screened with NY-ESO-1 18mer on day 13 after culture





T cells generated with DC+ISCOM/NY-ESO-1 and screened with 18mer peptides at day 13 after culture

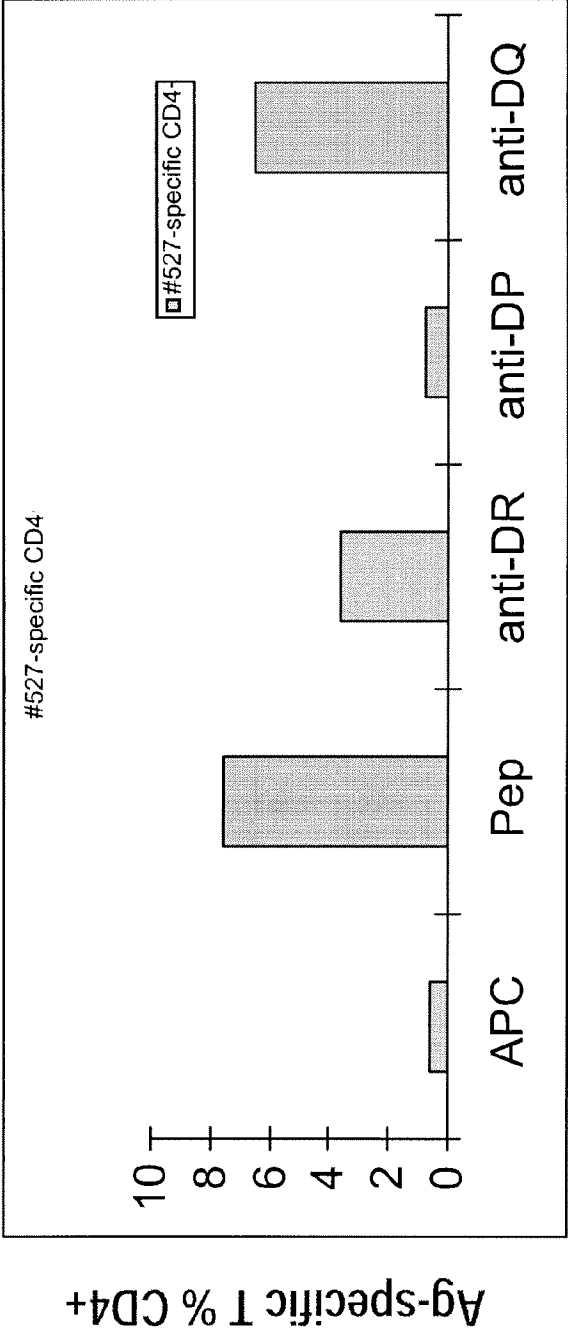


# Further characterisation of DC generated CD4 T cells

- Lines & clones established
- Antibodies
  - Anti DR, DP, DQ
- LCL lines
  - LCL auto: DR1, DR2, DP4
  - LCL 9080: DR1, ---, ---
  - LCL 9014: ---, DR2, ---
  - LCL T291: ---, DR2, DP4
  - LCL T282: ---, ---, DP4
- Tumor lines
  - NW38: DR1, ---, ---, NY-ESO-1(+)
  - LAR1a: ---, DR2, ---, NY-ESO-1(+)
  - SK-Mel 37: ---, ---, ---, NY-ESO-1(+)

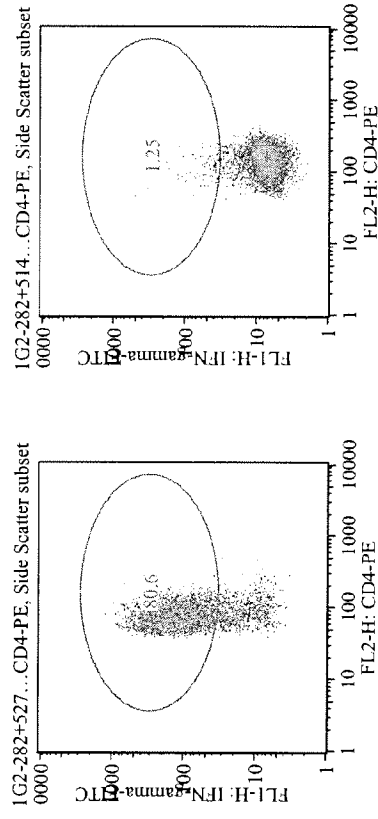
#527-specific CD4+ T cells are DP restricted

(DC stimulated then #527-pulsed BCL stimulated 2x)

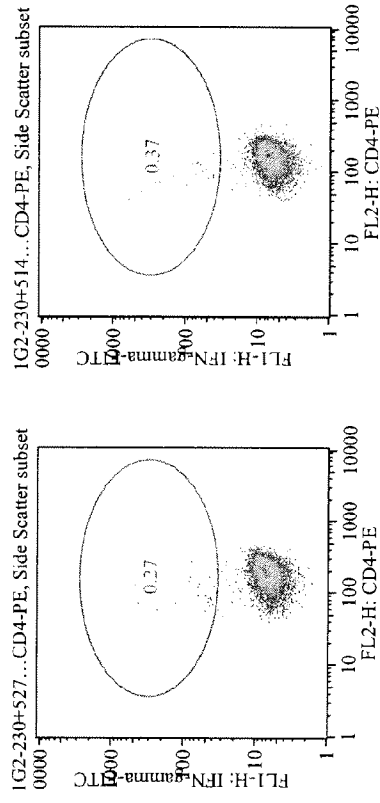


## #527-specific T cells are DP4 restricted

DP4+  
LCL  
(282)

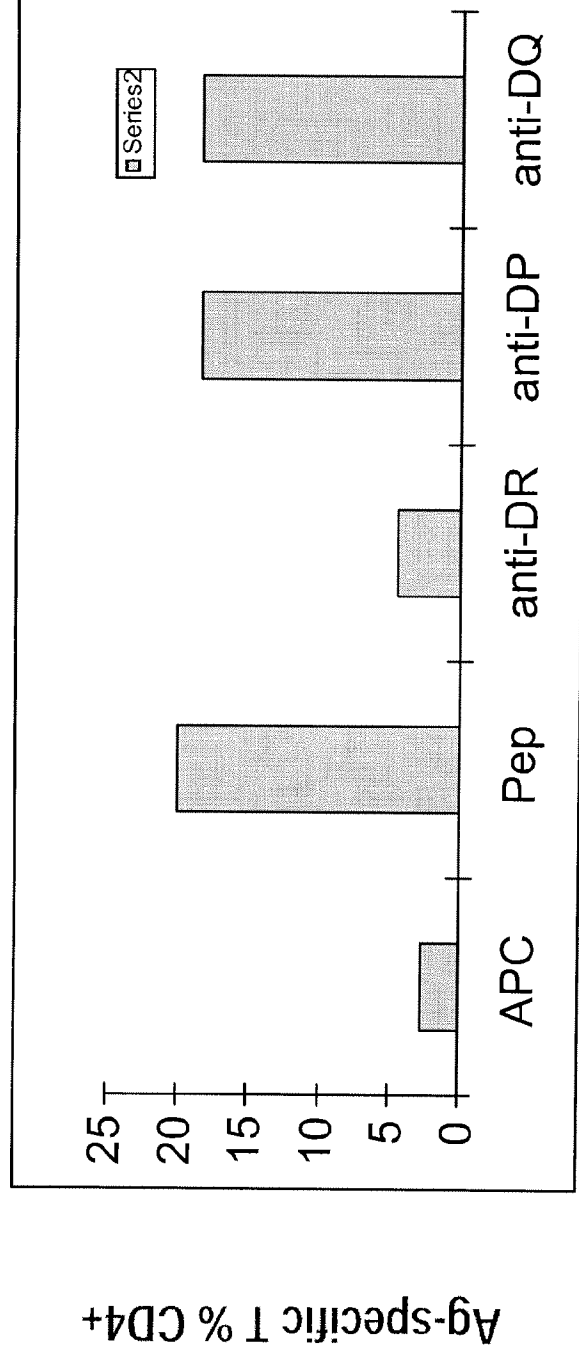


DP4-  
LCL  
(230)



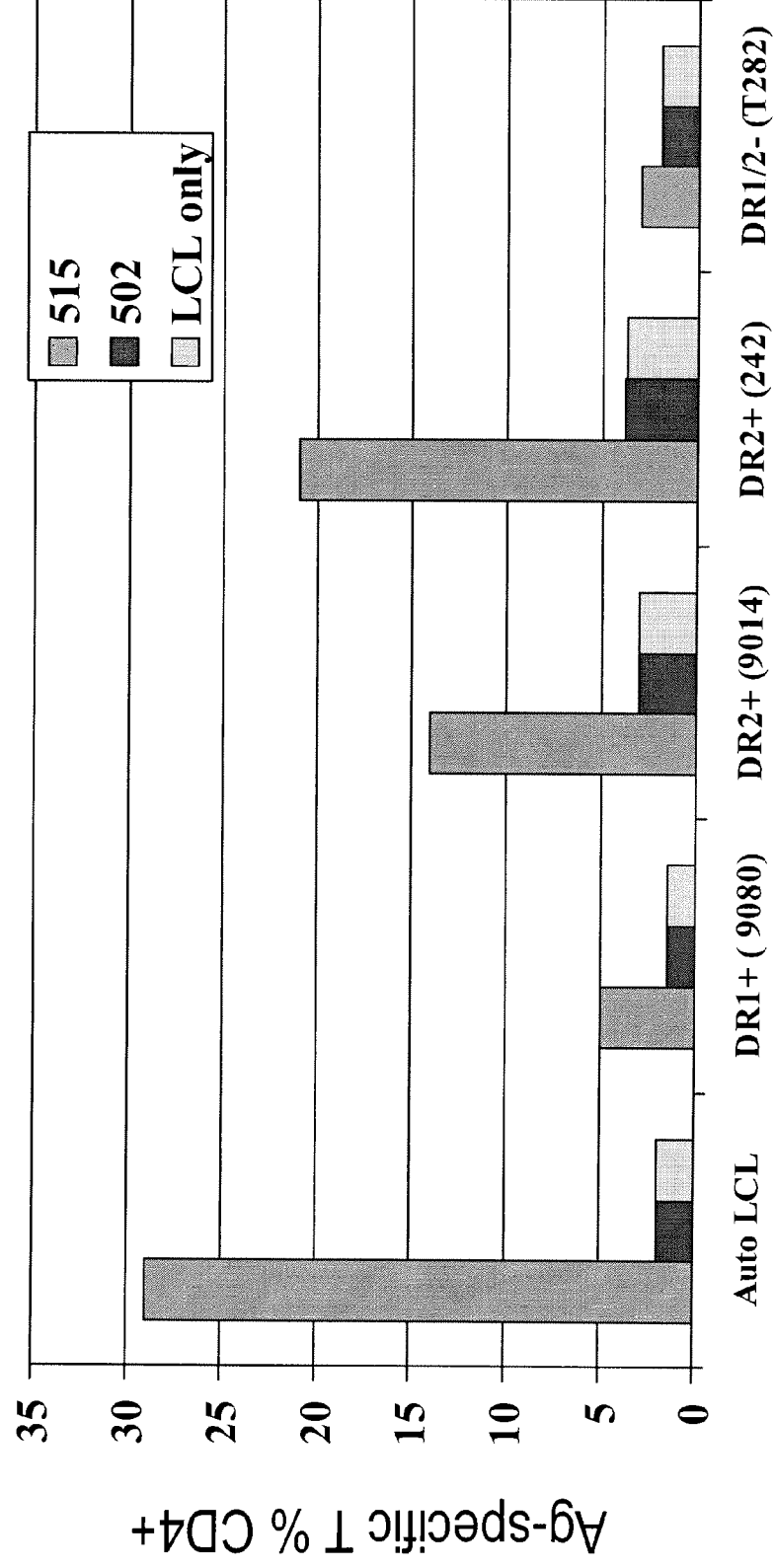
# #515-specific CD4+ T cells are DR restricted

(DC stimulated then #515-pulsed BCL stimulated 2x)



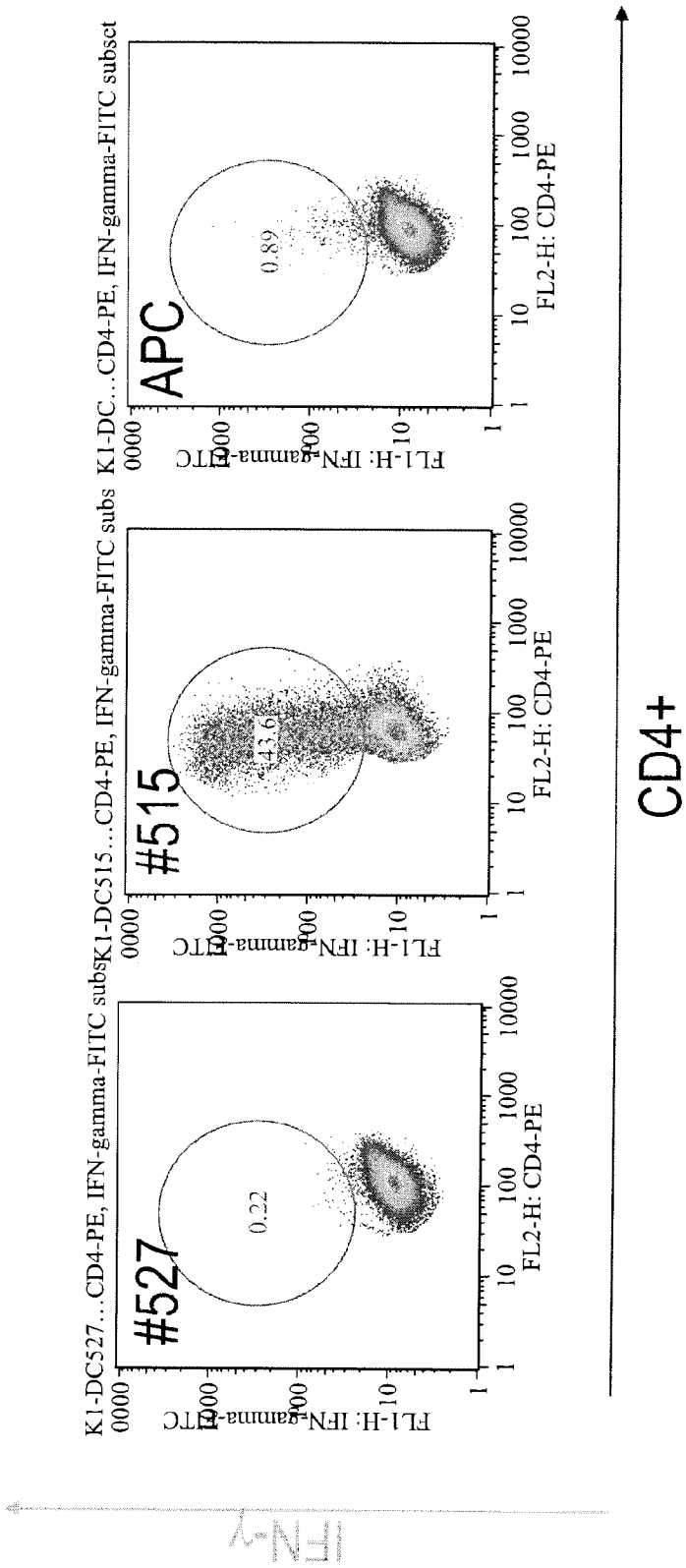
Treatments

# DR restriction of #515-specific T cells ( #515 2xstimulations )

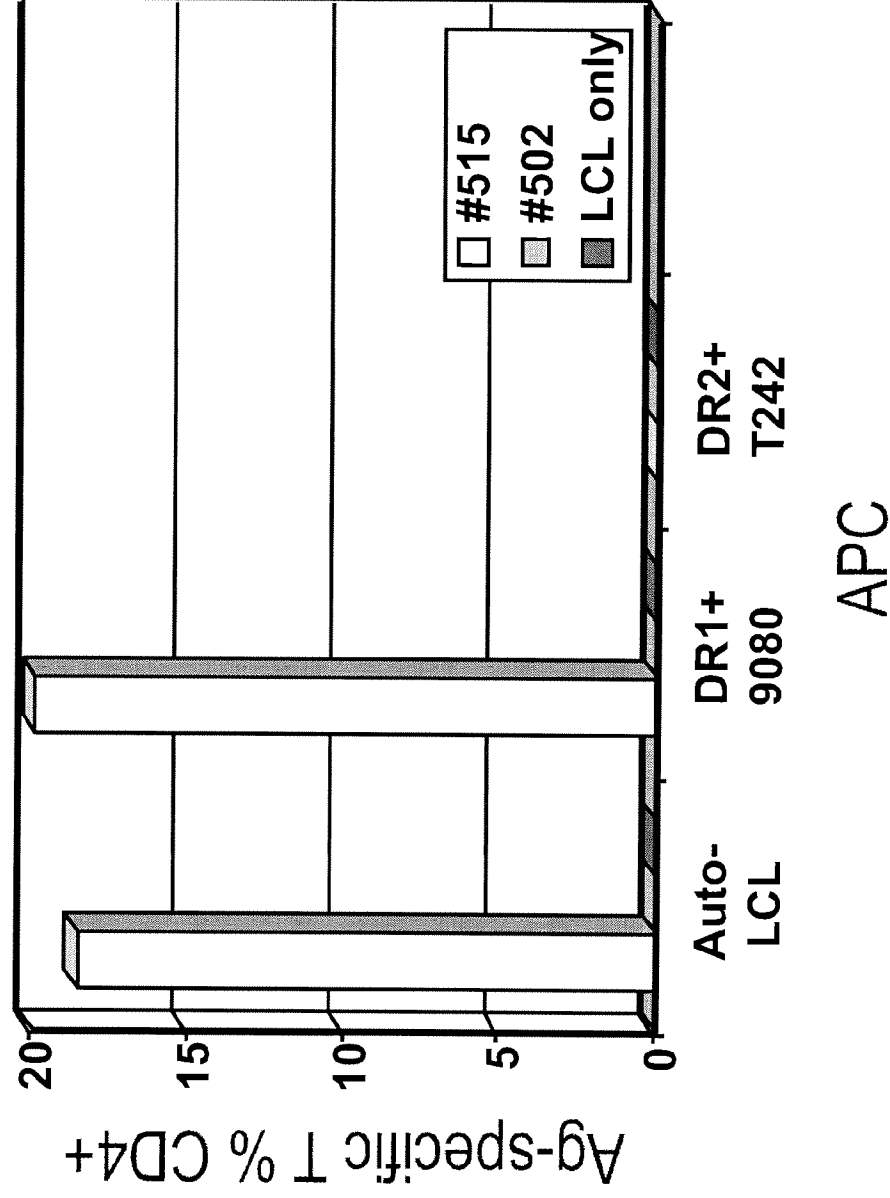


# #515-specific T clone K1

generated with #515-pulsed DC



T clone K1 is DR1-restricted





# Summary

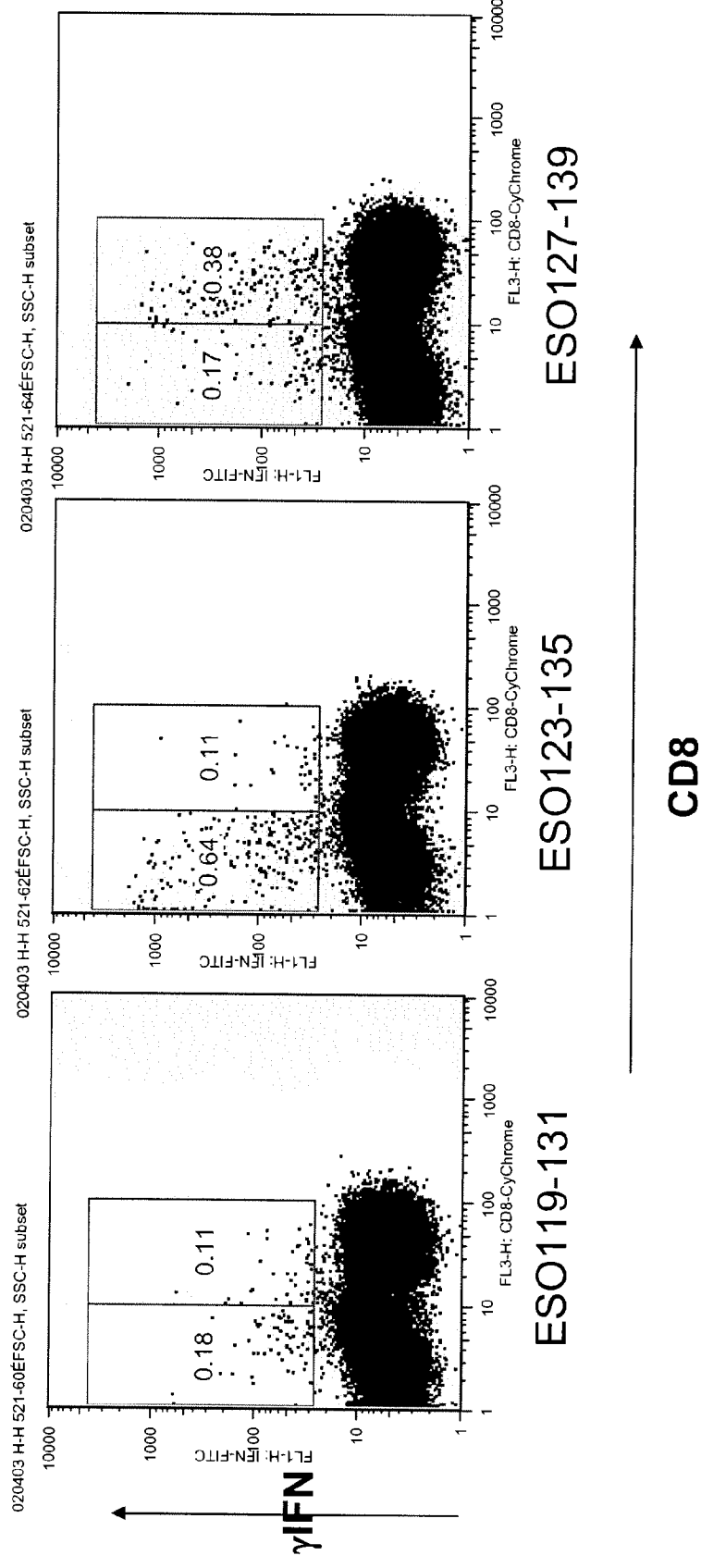
- Response identified to 3 probable Class II peptides
- Restricted by
  - DP4 (previously reported)
  - DR1
  - DR2
- Minimum epitope is being determined

# Simplified method for screening for CD4/CD8 response to ESO/ISCOM vaccine against peptide panels

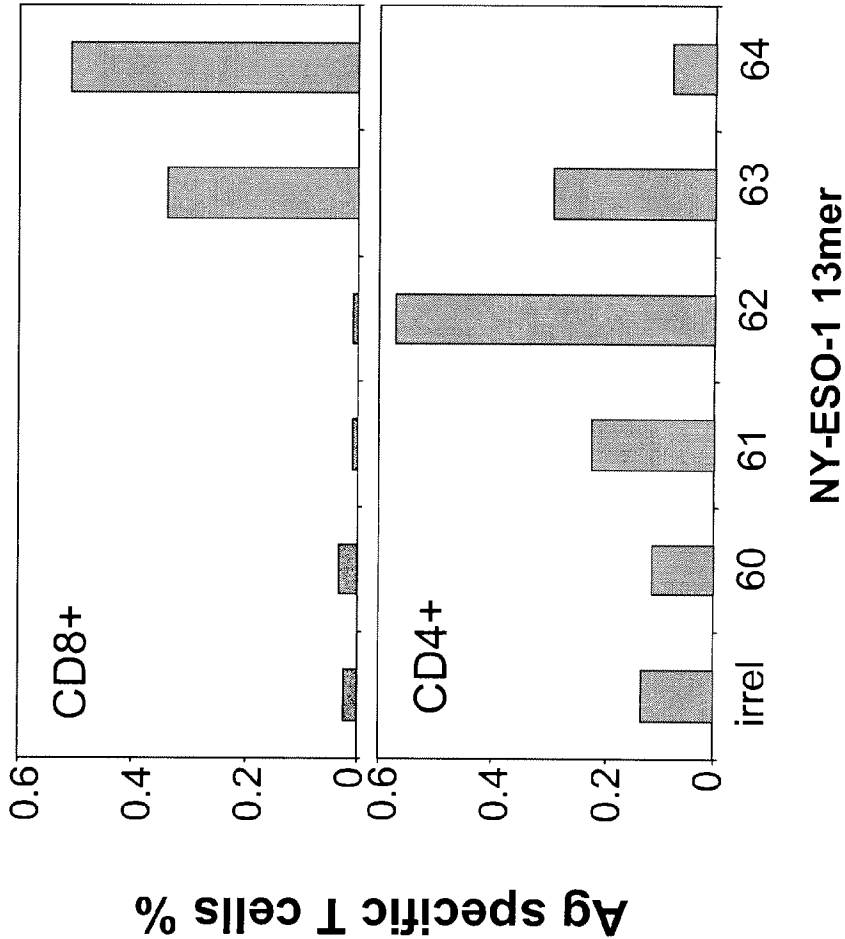
## Method

1. Frozen PBMC Day 86
2. Autologous cells used as APC
3. Bulk cultures stimulated with NY-ESO-1 18mer peptides
4. On day 7, cultures screened for intracellular cytokine staining (ICS) for IFN $\gamma$  against panel of 18mer peptides pulsed onto autologous PBMC.
5. Day 9: Positive cultures were further tested against a panel of shorter overlapping peptides (13mers)
6. Day 17: Confirmation assay: ICS performed again using the same 13mer peptides
7. All ICS were triple colour for CD8(CyCh), CD4(PE) and  $\gamma$ IFN-FITC.

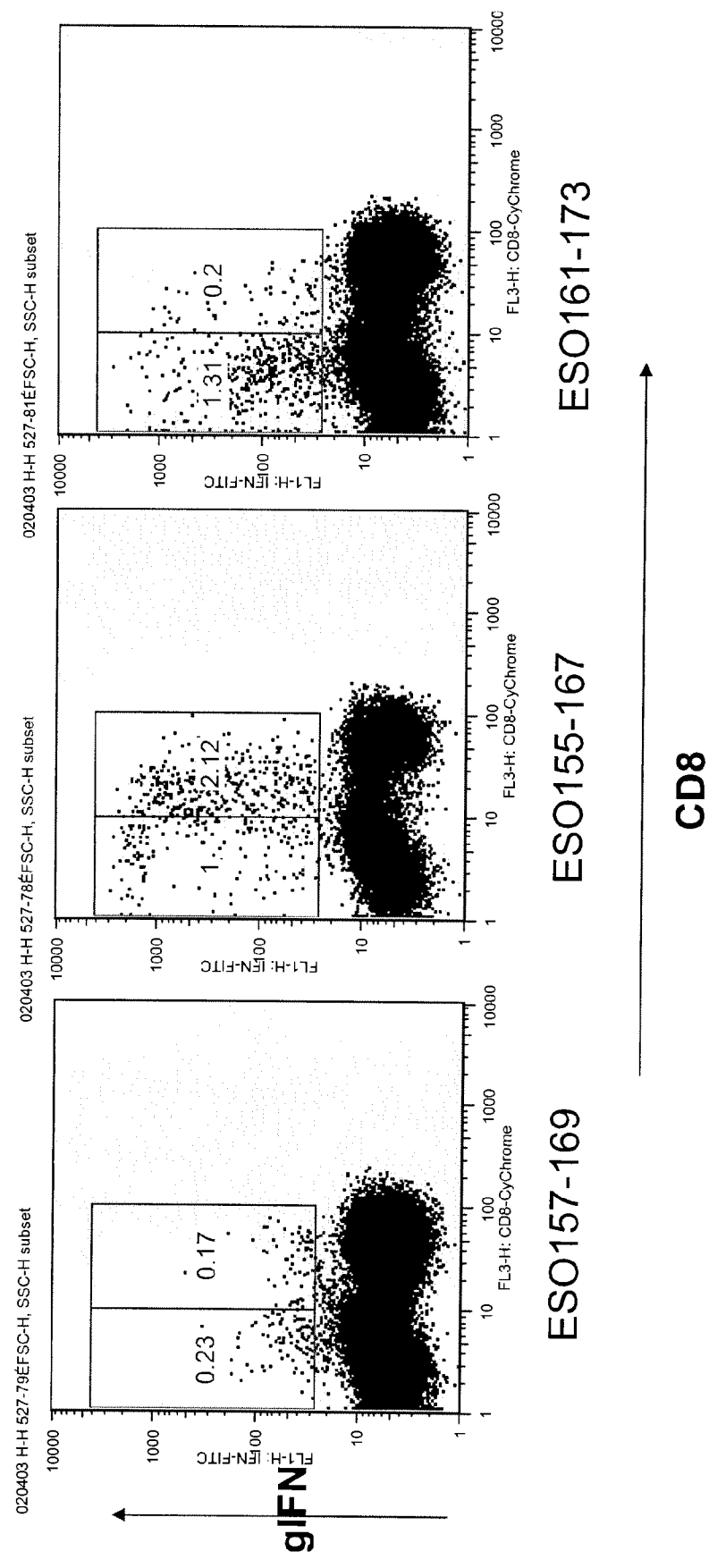
# Pt 107 CD4+CD8 Bulk culture screening #521 (ESO121-138) stimulated T cells



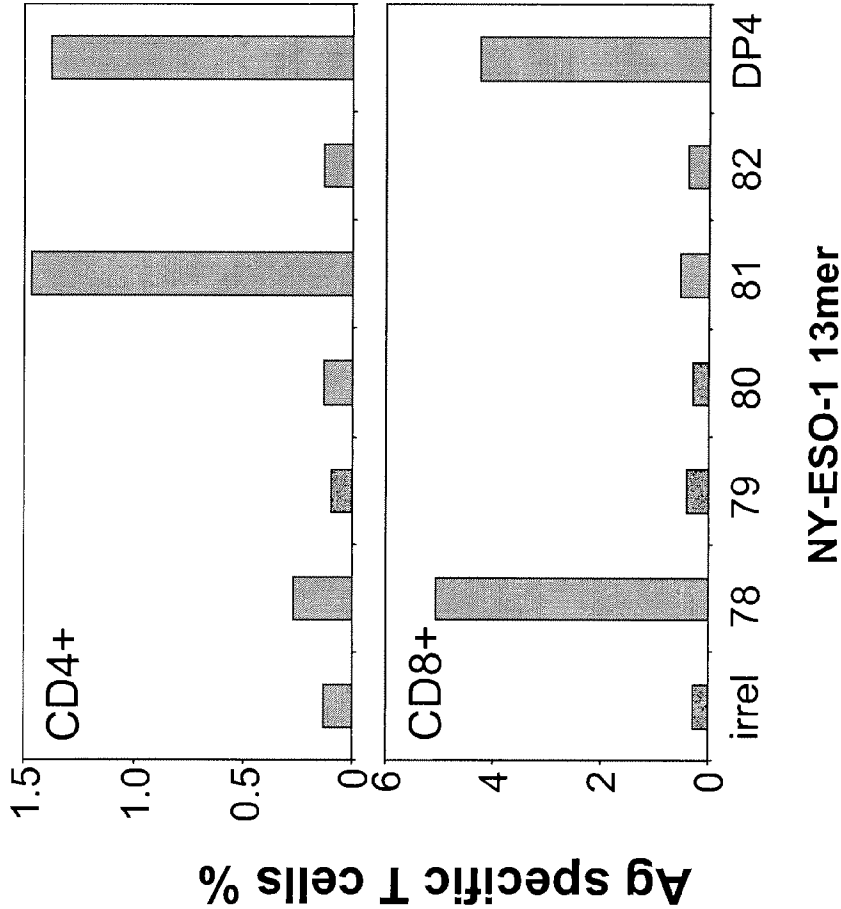
Pt 107 CD4+CD8 Bulk culture screening  
#521 (ESO121-138) stimulated T cells



# Pt 107 CD4+CD8 Bulk culture screening #527 (ESO157-174) stimulated T cells



Pt 107 CD4+CD8 Bulk culture screening  
#527 (ESO157-174) stimulated T cells



ESO-1(139-156)	AADHRQLQLSISSCLQQL	(peptide #524)
ESO-1(145-162)	LQLSISSCLQQLSLLMWI	(peptide #525)
ESO-1(151-168)	SCLQQLSLLMWITQCFLP	(peptide #526)
ESO-1(157-174)	SLLMWITQCFLPVFLAQP	(peptide #527)
ESO-1(163-180)	TQCFLPVFLAQPPSGQRR	(peptide #528)

### Previously described Epitopes

HLA DP4

SLLMWITQCFLPVF

HLA-A2

ESO-1a(157-167)

SLLMWITQCFL

ESO-1b(157-165)

SLLMWITQC or SLLMWITQV

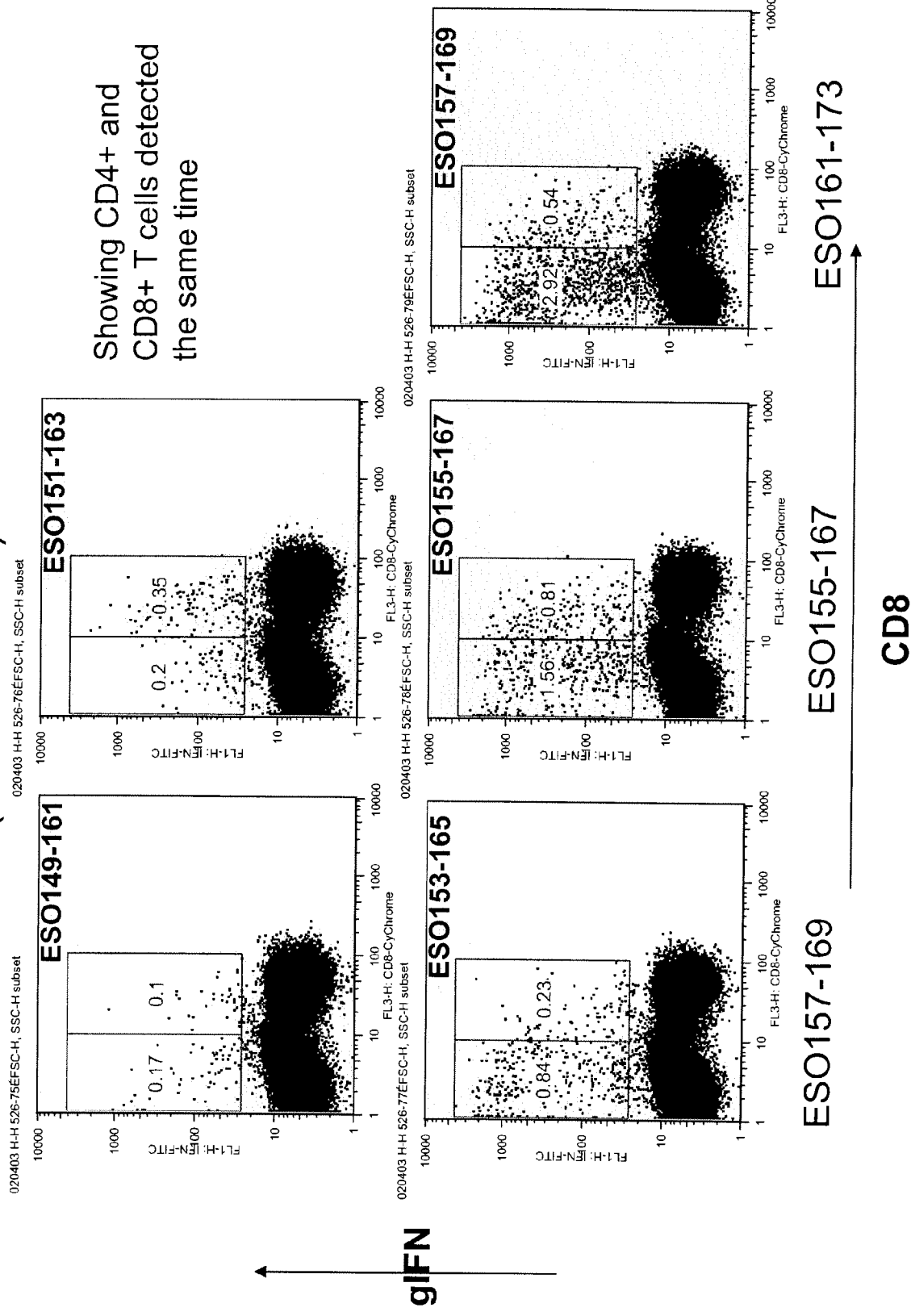
ESO-1c(155-163)

QLSLLMWIT

ESO-1d(159-162)

LLMWITQCF

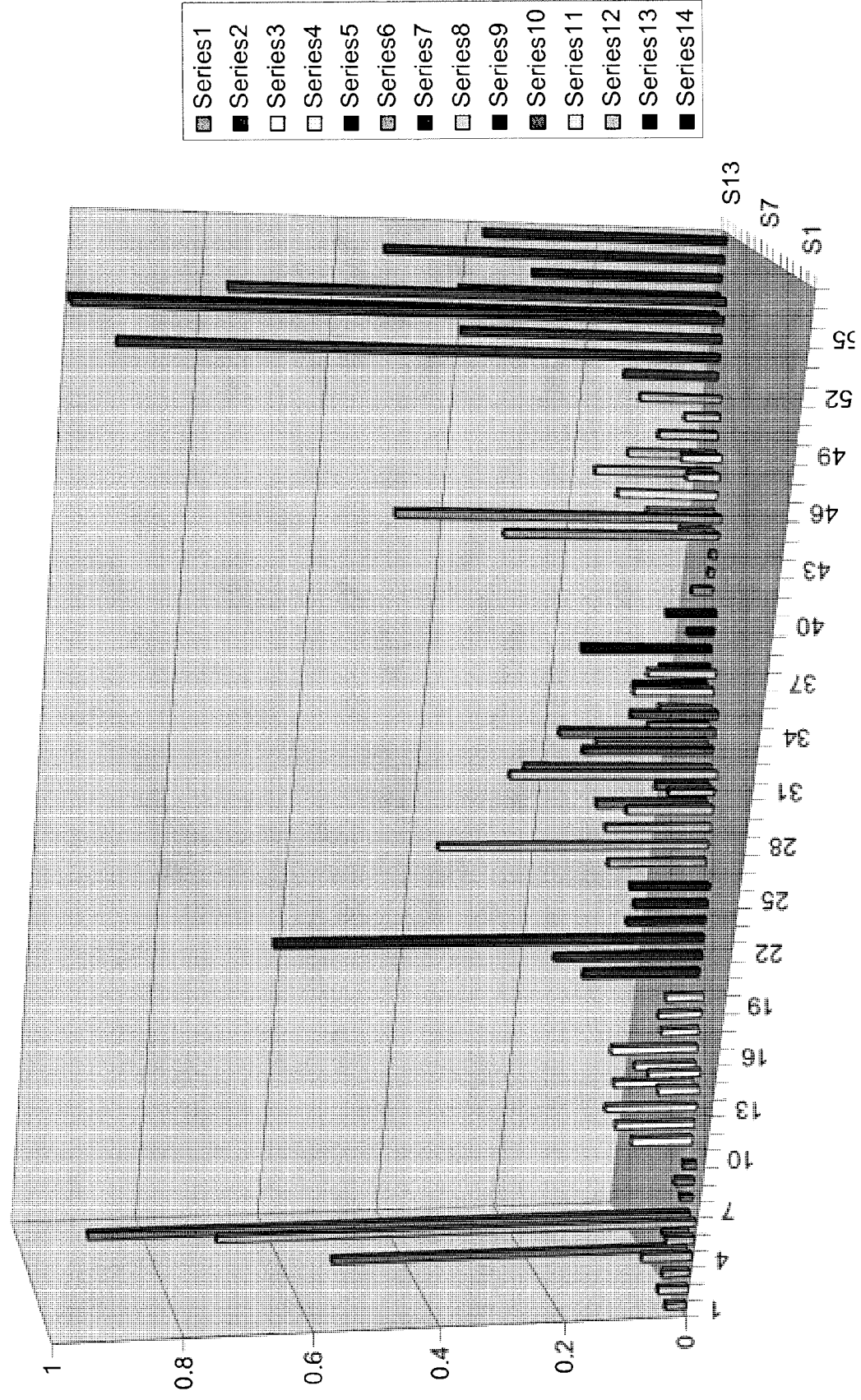
# Pt 107 CD4+CD8 Bulk culture screening #526 (ESO151-168) stimulated





# Pt 107

HLA typing: A1, A2, B8, B27, Bw4, C\*0102, C\*07011/012/06, DRB1\*0101 (DR1),  
 DRB1\*1501 (DR15), DPB1\*0401 (DP4), DQB1\*0501 (DQ5), DQB1\*0602 (DQ6).



# Immunology Conclusions

- Screening for immune reactivity has been successful using a simplified methods with autologous PBMC & panels of overlapping peptides
- Ongoing work will define minimal epitopes and HLA class I and II restriction for each
- For patients tested to date there is clear evidence of a broad-based CD4 and C8 cellular immune response against NY-ESO-1 in addition to antibody responses
- We have reduced concern that contaminating bacterial protein may have dominated immune responses to this vaccine
- PBMC from Pts in this trial should make it possible to map the majority of NY-ESO-1MHC I & II epitopes.

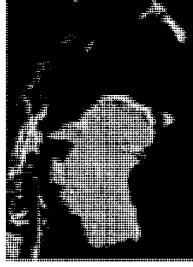
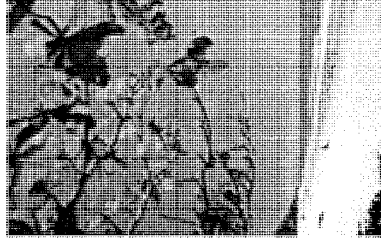
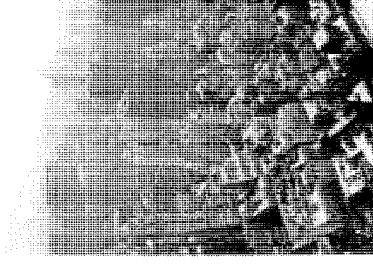
# Future Directions for NY-ESO-1 ISCOM Vaccine

Clinical responses to vaccination need to be optimised.

- Lab studies
  - Further studies to map epitopes will make it possible to
    - Investigate impact of Class II determinants on class I response
    - Investigate immunodominance in a human cancer antigen system
- Clinical Directions:
  - Optimization of the vaccine
    - Route, schedule etc
    - Other (cytokines, anti-CTLA4)
  - Evaluate clinical impact
    - Patients with evaluable disease
      - What are the determinants of clinical response?
      - Prospectively identify potential clinical responders
    - Adjuvant therapy of NY-ESO-1 +ve tumours

# Future Directions

- Optimising clinical strategies
- Building collaborative networks
  - Ludwig Institute International Trials Program
  - Cancer Vaccine Collaborative (New York)
    - MSKCC, Cornell, NYU, Sinai, Columbia, Roswell Park
  - Cancer Vaccine Collaborative (South Pacific)



# Acknowledgements – Tumour Antigen studies

## Anatomical Pathology

Duncan MacGregor

Sue Sturrock

## Cancer Vaccine Lab

Hilary Vaughan

Susan Svobodova

Fiona St Clair

## New York

Achim Jungbluth

Lisa Stockert

Yao Chen

Matt Scalan

## Victorian Transplantation and Immunogenetics Service (VTIS)

Brian Tait & Carmel Kanaan in  
collaboration with Soldano Ferrone

# Acknowledgements – Clinical Trial

## ARMC - Oncology

Ian Davis  
Mark Shackleton  
Phil Parente  
T-cell Laboratory  
Weisan Chen  
Quiyan Chen  
Heather Jackson  
Eugene Maraskovsky  
Mark Rizkalla  
Tsin Tai  
Kelly-Anne Masterman

## CDCT

Grant MacArthur  
Michael Green  
Richard Fox

## ARMC-BPF

Andrew Scott  
Roger Murphy  
Mike Rubira  
Glen Cartwright  
Jeff Rood

## Trials Centre

Wendy Hopkins  
Heather Goldie  
Sharen Gibbs  
Julie Newton

## CSL

Simon Green  
Lena Miloradovic  
Andrew Cuthbertson  
Darryl Maher  
David Ryan  
Michael McNamara  
Debbie Drane

## New York

Lloyd Old  
Eric Hoffman  
Gerd Ritter  
Sacha Gnjatich  
Yao Chen  
Lisa Stockert  
Lisa Pugliese